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كلية الدراسات العليا

التاريـــخ: / /

نموذج رقم (١٦)
اقرار والتزام بالمعايير الأخلاقية والأمانة العلمية وقوانين الجامعة الأردنية وأنظمتها وتعليماتها لطلبة الدكتوراه

أنا الطالب: منال ممدوح الخزاوي الرقم الجامعي: (٩٠٦٠٢٣٩)
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عنوان الاطروحة: ....
The synthesis of novel heterocyclic carboxamide derivatives and evaluation of their antihyperlipidemic and antioxidant activity.

اعلن بأنني قد التزمت بقوانين الجامعة الأردنية وأنظمتها وتعليماتها وقراراتها السارية المفعول المتعلقة باعداد اطروحات الدكتوراه عندما قمت شخصيا" باعداد اطروحتي وذلك بما ينسجم مع الأمانة العلمية وكافة المعايير الأخلاقية المتعارف عليها في كتابة الأطروحات العلمية. كما أنني أعلن بأن اطروحتي هذه غير منقولة أو مستلة من أطاريح أو كتب أو أبحاث أو أي منشورات علمية تم نشرها أو تخزينها في أي وسيلة اعلامية، وتأسيسا" على ما تقدم فانني أتحمل المسؤولية بأنواعها كافة فيما لو تبين غير ذلك بما فيه حق مجلس العمداء في الجامعة الأردنية بالغاء قرار منحي الدرجة العلمية التي حصلت عليها وسحب شهادة التخرج مني بعد صدورها دون أن يكون لي أي حق في التظلم أو الاعتراض أو الطعن بأي صورة كانت في القرار الصادر عن مجلس العمداء بهذا الصدد.

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THE SYNTHESIS OF NOVEL HETEROCYCLIC CARBOXAMIDE DERIVATIVES AND EVALUATION OF THEIR ANTIHYPERLIPIDEMIC AND ANTIOXIDANT ACTIVITY

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I Dedicate this thesis

To my Precious children

Shadi

Ramzi

Diala

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LIST OF ABBREVIATIONS AND SYMBOLS

ABCA	: ATP-Binding Cassette Transporter.
ACAT	: Acyl Co-A Cholesterol Acyl Transferase.
AGEs	: Advanced Glycation End-products.
AP-1	: Activator Protein-1.
BAS	: Bile Acid Sequestrants.
BF	: Benzafibrate.
BuLi	: Butyl lithium.
$^{\circ}C$	: Degree Celsius.
CAT	: Catalase.
$CDCl_3$	: Deuteriated Chloroform.
CE	: Cholesterol Ester.
CERP	: Cholesterol Efflux Regulatory Protein.
CETP	: Cholesterol Ester Transfer Protein.
CG	: Control Group.
$CHCl_3$	: Chloroform.
CHD	: Coronary Heart Disease.
CMs	: Chylomicrons.
DCC	: 1,3-dicyclohexylcarbodiimide.
DCM	: Dichloromethane.
DM	: Diabetes Mellitus.
DMF	: N,N-dimethylformamide.
DMSO	: Dimethylsulfoxide.
DPPH	: 1,1-diphenyl-2-picrylhydrazyl.
EA	: Elemental Analysis.

ESI	: Electrospray Ionization.
EtOAc	: Ethylacetate.
g	: Grams.
GPCR's	: G-protein Coupled Receptors.
G6PD	: Glucose 6 Phosphate Dehydrogenase.
GPx	: Glutathione Peroxidase.
GR	: Grignard Reagent.
HDL-C	: High Density Lipoprotein Cholesterol.
HG	: Hyperlipidaemic Group.
HLGPa	: Human Liver Glycogen Phosphorylase a.
HMG-CoA	: 3-hydroxy-3-methyl-glutaryl-coenzyme A.
4-HNE	: 4-Hydroxynonenal.
H_2O_2	: Hydrogen Peroxide.
HRMS	: High- Resolution Mass Spectra.
hrs	: Hours.
HTGL	: Hepatic Triglyceride Lipase.
IDL	: Intermediate Density Lipoprotein.
i.p.	: Intraperitoneal.
IR	: Infra Red.
KBr	: Potassium Bromide.
LCAT	: Lecithin Cholesterol Acyl Transferase.
LD	: Lethal Doase.
LDL-C	: Low Density Lipoprotein Cholesterol.
LPL	: Lipoprotein Lipase.
MAO	: Monoamine Oxidases.

MDA	: Malondialdehyde.
MeOH	: Methanol.
mg	: Milligrams.
$MgSO_4$	: Magnesium sulfate.
mmol	: Millimole.
m.p.	: Melting point.
MTP	: Microsomal Triglyceride Protein.
NAD	: Nicotinamide Adenine Dinucleotide.
$NaOC_2H_5$	: Sodium ethoxide.
NaOH	: Sodium hydroxide.
NF-κB	: Nuclear Factor-κB.
NMR	: Nuclear Magnetic Resonance.
NPC1L1	: Niemann Pick C1-Like 1.
NSAIDs	: Non-Steroidal Anti-Inflammatory Drugs.
$O_2^{\cdot}$	: Superoxide anion.
$-OH^{\cdot}$	: Hydroxyl radicals.
PCG	: Positive Control Group.
PLTP	: Phospholipid Transfer Protein.
PPAR	: Peroxisome Proliferator Activated Receptor.
PPREs	: Peroxisome Proliferator Response Elements.
RCT	: Reverse Cholesterol Transport.
R_f	: Retention factor.
RNS	: Reactive Nitrogen Species.
$ROO^{\cdot}$	: Lipid Peroxide radicals.
ROS	: Reactive Oxygen Species.

RT	: Room Temperature.
SAR	: Structure Activity Relationship.
SEM	: Standard Error of the Mean.
$SOCl_2$	: Thionyl chloride.
SOD	: Superoxide Dismutases.
SR-BI	: Scavenger Receptor class B, type I.
TEA	: Triethylamine.
TG	: Triglyceride.
THF	: Tetrahydrofuran.
TLC	: Thin Layer Chromatography.
TMS	: Tetramethylsilane.
TZD	: Thiazolidinedione.
VLDL	: Very Low Density Lipoprotein.
WHO	: World Health Organization.

THE SYNTHESIS OF NOVEL HETEROCYCLIC CARBOXAMIDE DERIVATIVES AND EVALUATION OF THEIR ANTIHYPERLIPIDEMIC AND ANTIOXIDANT ACTIVITY

By

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Abstract

This research aimed to prepare novel indole-2-carboxamide derivatives (series **A-G**) and counterparts pyrrole and furan-2-corboxamides (series **H**) and to evaluate their *in vitro* antioxidant activity at concentration equal to 10 µg/ml and *in vivo* hypolipidemic activity in Triton WR-1339 animal rat model at a fixed concentration equal to 57 µM.
The novel compounds prepared are *1H*-indole-2-carboxamide derivatives of: benzophenones (series **A**; **6, 8, 10, 12, 14, 16**); anthraquinones (series **B**; **18, 20, 22, 24, 26**); acetophenones (series **C**; **28, 30, 32, 34, 36, 38**); benzene-aniline (series **D**; **39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63**); benzyl and phenylethyl (series **E**; **65, 67, 69, 71, 73, 75**) and heterocyles (series **F**; **77, 79, 81, 83**); substituted indole (series **G**; 5-Cl: **88, 89, 90, 93; 5-F: 93** and **N-Me: 96, 97, 98**); furan and pyrrole-carboxamide derivatives (series **H**; **pyrrole: 101, 102, 103, 104, 105, 108; furan: 110, 111**).
Most of these compounds were prepared adopting the direct coupling of the ethyl indole-2-caroxylate ester or indole acyl chloride with the correspondent amines in DMSO, DMF or $CHCl_3$ with or without a base catalyst. Alternatively, direct fusion was utilized with some groups giving better yields. Most compounds were obtained in a reasonable yield and their structures were confirmed by Nuclear magnetic resonance spectra (NMR), Infra red (IR), High resolution mass spectra (HRMS) and Elemental analysis (EA).

For anti-hyperlipidemic activity, the dose was chosen based on acute toxicity study of model compound **6**. Acute hyperlipidemia was induced by administration of single dose of Triton WR 1339 (250 mg/kg, i.p) to rats. The degree of protection of each tested

compound was determined by measuring levels of serum TG, TC, HDL-C and LDL-C levels after 8 hours against Bezafibrate (100 mg/kg p.o) as reference standard.

Compounds **6** and **10** from series A, have shown a significant reduction in triglyceride levels (71%) compared to hyperlipidemic rats. Apart from **20,** all compounds **18, 22, 24** and **26**, have shown a significant reduction in triglyceride levels (65-71%) compared to hyperlipidemic rats. Novel compounds from (Series C, D, E and F), were all not able to reduce TG levels of hyperlipidemic rats. Compounds **93, 88** and **90** from Series G, have shown a significant reduction in triglyceride levels (70-82%) compared to hyperlipidemic rats. Novel compounds **110, 103, 104** and **111** from Series H, have also shown a significant reduction in triglyceride levels (67-71%) compared to hyperlipidemic rats.

The reduction of total cholesterol by compounds **6, 18, 24, 26, 36, 88, 90, 103, 104** and **111**, was associated with a decrease of its LDL fraction, which is the target of several hypolipidemic drugs.

In addition, compounds **6, 10, 18, 22, 24, 26, 36, 38, 93, 88, 90, 110, 103, 104** and **111,** have increased HDL level, which is known for its preventive role against atherogenesis.

Promisingly, the reduction of lipid profile by compounds **6, 10, 18, 22, 24, 26, 36, 38, 93, 88, 90, 110, 103, 104** and **111**, at 57 μM after 8 hours of Triton injection is more significant than the reduction induced by Bezafibrate.

Indole structure or its isoster is required to afford a H-bond donor or acceptor of the hit to bind with the receptor. A lipophilic moiety which is represented by two phenyl group fused or apart is required to possibly participate in π– π stacking with aromatic amino acids residue of the receptor and add in the lipophilicity of the compound as proved from compounds **36** and **38**.

Compounds **22, 57, 30, 55** and **59** have shown excellent percent inhibition of DPPH activity (455%, 226%, 166%, 122% and 53% respectively) and were the most effective DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavengers. Compound **22**, showed a significant increase in the radical scavenging activity in addition to excellent dyslipidemic activity, which could be a promising candidate for a novel compound with dual activity.

1. Introduction:

Hyperlipidemia and thereby atherosclerosis is the leading cause of cardiac illness and death (Farnier and Davignon, 1998). In 1983 it was demonstrated for the first time that there exists a link between serum cholesterol levels and the risk to coronary heart disease (CHD) (Grundy *et al*., 2004). World Health Organization (WHO) reports that high blood cholesterol contributes to approximately 56% cases of CHD worldwide and causes about 4.4 million deaths each year (Batra *et al*., 2000; Pooja and Priscilla, 2009). Data are now accumulating on the effects of lowering serum triglyceride levels in improving coronary risk. More studies are still required, but metabolic studies indicate that high serum triglycerides are a marker for the presence of atherogenic small dense low-density lipoproteins. Low concentrations of high density lipoprotein cholesterol is also a marker for coronary risk, but the case for increasing levels by drugs is unclear. There is evidence that combined hyperlipidemia (i.e. elevated low density lipoprotein cholesterol (LDL-C) and triglyceride (TG) promotes CHD to a significantly greater extent than either high LDL-C or TG alone. Triglyceride / high density lipoprotein cholesterol (HDL-C) ratio, as a relatively novel lipoprotein index, indicates the presence of small dense LDL particles and could serve as a good predictor of CHD. A low TG/HDL-C ratio is indicative of primarily large, nonatherogenic LDL particles, whereas a high TG/HDL-C ratio indicates a larger population of small, dense pro-atherogenic LDL particles. As a result of this interrelation between TG and HDL-C, recent focus on high TG- low HDL-C abnormality has grown considering risk assessment and drug therapy for CHD (Miller *et al*., 2008; Hadaegh *et al*., 2009). The term “dyslipidaemia” nowadays is increasingly being used to describe abnormal changes in lipid profile, replacing the old term hyperlipidaemia (Elnasri and Ahmed, 2008). CHD is caused by the narrowing of the artery that supplies nutrients and

oxygen to the heart. The main reason for this narrowing is atherosclerosis. Atherosclerosis is a disorder of the arterial wall characterized by accumulation of cholesterol ester (CE) in cells derived from the monocyte-macrophage line, smooth muscle cell proliferation and fibrosis, and results in narrowing the blood vessels. The etiology and pathogencity of CHD lie in the casual relationship between the development of atherosclerosis, elevated plasma lipid percentage cholesterol levels in blood and plasma, genetic makeup, endocrinological aberration, immunologic and autonomic factors, blood flow and coagulation. High serum LDL-C and elevated total cholesterol levels are the most prevalent indicators for susceptibility to atherosclerotic heart disease (Fruchart *et al*., 2008).

Recent studies have demonstrated that increased formation of free radicals/reactive oxygen species (ROS) contribute to CHD progression. ROS induce cardiac dysfunction and cardiac apoptosis and/or necrosis in heart failure. ROS are formed intracellularly and are controlled by antioxidant defense. The generation of large amounts of ROS can overwhelm the intracellular antioxidant defense, causing activation of lipid peroxidation, protein modification and DNA breaks. ROS induced depletion of antioxidants is a key factor for the initiation of atherosclerosis and the development of CHD (Pooja and Priscilla, 2009).

To reduce the rate of mortality, it is therapeutically recommended to undergo diet or/and drug therapy to lower lipid levels within the normal range. Therapy of hyperlipidemia merits the consideration in the established atherosclerotic state. Allopathic hyperlipidemia drugs are available at large scale in the market; these drugs have different mechanisms of action and variable efficacy depending on the lipid profile of an individual. In spite of their lipid-lowering effect, the side effects and

contraindications of these drugs have marred their popularity (Batra *et al*., 2000). Thus, research is still pursuing to find out novel agents that are more effective and safe.

1.1. Major families of blood (plasma) lipoproteins:

Lipids are carried in the blood in the form of lipoprotein particles with TG and CE carried in the interior and phospholipid, free cholesterol and apolipoproteins at the surface (Figure 1). Apolipoproteins are the proteins that carry lipids in plasma; making the lipoproteins soluble in plasma and thereby acting as interface between plasma and the core components. Apolipoproteins also play an important role in stabilizing the structure of protein and act as ligands for specific receptors, having physiological role in lipoprotein metabolism (Jain *et al*., 2007).

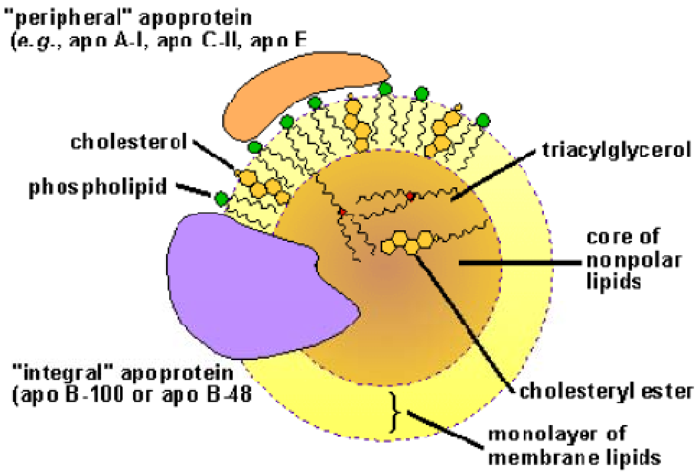


Figure 1. General structure of lipoprotein

Depending on their lipid carrying capacity lipoproteins are classified into:

I. Chylomicrons:

Chylomicrons (CMs) are the least dense of the lipoproteins and do not migrate in an electric field (Gotto and Pownall, 2003). There are three stages in the chylomicron's "life cycle": nascent CM, mature CM and CM remnant. The nascent CMs are formed in

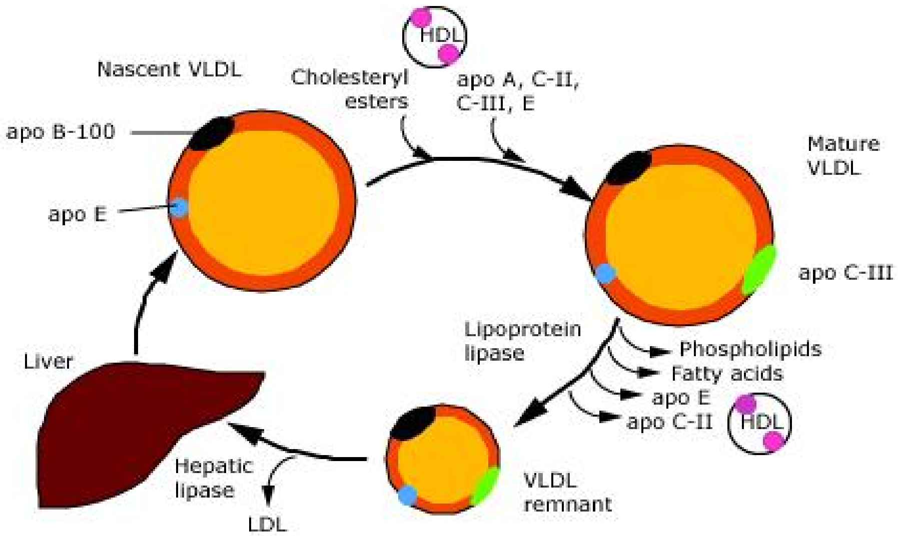
intestinal mucosa during solubilization of dietary fats by bile acids, its main apolipoprotein component is apo B-48. CMs exchange components with HDL, while circulating in lymph and blood. The HDL donates apo C-II and apo E to the nascent CM and thus converts it to a mature CM. Once triglyceride stores are distributed, the CM returns apo C-II to the HDL; but keeps apo E and thus, becomes a CM remnant. CMs are metabolized in the liver resulting in the formation of very low density lipoprotein (VLDL) which in turn is catabolised to form intermediate density lipoprotein (IDL), which is further catabolised to LDL (Jain *et al*., 2007).

The apo C-II in CMs activates lipoprotein lipase (LPL) in the presence of phospholipids. This LPL removes fatty acids of CMs from TGs. A progressive decrease in particle diameter occurs as TGs in the core are depleted and small apoproteins are transported to HDL. The resultant CMs remnants are taken up by receptor-mediated endocytosis, as into hepatocytes (Chung and Wasan, 2004). The concentration of CMs can be controlled only by reducing dietary fat consumption. There is no current therapeutic approach that will enhance CM catabolism except for insulin replacement in patients with type–I diabetis. CMs function to deliver dietary TGs to adipose tissue and muscle and dietary cholesterol to liver.

Chylomicrons contain several apoproteins, including apo B48, Apo E and Apo C-II. Apo B48 is unique to CMs, apo C-II activates lipoprotein lipase resulting in fatty acid release to heart, skeletal muscles and mammary glands. The presense of apo E facilitates the clearance of chylomicron remnants by the liver (Chung and Wasan, 2004).

II. Very Low Density Lipoproteins (VLDL):

Very low density lipoproteins are the next in size to CMs, which are synthesized in the liver and transport TG and CE from the liver into the blood stream to other parts. VLDLs are composed mainly of TG, yet are more enriched in CE than are chylomicrons. Like nascent CMs, newly released VLDL acquires apo CII and apo E from circulating HDLs (Jain *et al*., 2007). LPL located on capillary endothelial cell surface catalyses the release of fatty acids portion of VLDL to adipose tissue and muscle in the same way as CMs.



Apo E plays an important role in the metabolism of TG rich lipoproteins CMs, CM remnants, VLDL and IDL and has number of functions related to the binding and uptake of plasma proteins (Chung and Wasan, 2004).

III. Intermediate Density Lipoproteins (IDL):

Intermediate density lipoproteins are formed from VLDLs with the removal of TGs. They are also called (VLDL remnants) and may be scavenged by the liver (apo E) or may pick up cholesterol from HDL. As they acquire cholesterol, they become LDL. Conversion of IDLs to LDLs occur as more TGs are removed. The liver takes up IDLs

after they have interacted with the LDL receptor to form a complex, which is endocytosed by the cell. For LDL receptors to recognize IDLs requires the presence of both apo B-100 and apo E (the LDL receptor is also called the apo B-100/apo E receptor). Apo E is important in cholesterol uptake by LDL receptors. The transfer of cholesterol ester from HDL to IDL is mediated by cholesterol ester transfer protein (CEPT) (Chung and Wasan, 2004; Jain *et al*., 2007).

IV. Low Density Lipoprotein (LDL):

Low density lipoprotein is generated from VLDLs and IDLs by the action of LPL, thus increasing the relative proportion of cholesterol esters in the neutral core. The major function of LDL is to transport cholesterol to the extra hepatic tissues where it is taken up by the receptor-mediated endocytosis. The uptake of LDLs occurs predominantly in liver 75%, adrenals and adipose tissue. LDLs is the primary plasma carriers of cholesterol for delivery to all tissues. It contain 60-70% total cholesterol (Jain *et al*., 2007).

The LDL particle retains only apo B100 and the uptake of LDL by cells is initiated by the interaction of apo B100 with LDL receptors on the plasma membranes. The endocytosed membrane vesicles endosomes fuse with lysosomes, in which the apoprotein are degraded and the cholesterol esters hydrolyzed to yield free cholesterol (Costet, 2010). The cholesterol is then incorporated into the plasma membranes as necessary. Excess intracellular cholesterol is re-esterified by acyl Co-A cholesterol acyl transferase (ACAT), for intracellular storage (Chung and Wasan, 2004). LDL often called as “bad” cholesterol because LDL easily adheres to the wall of arteries, which leads to initiation of atherogenic process.

V. High Density Lipoproteins (HDL):

High density lipoproteins are synthesized by the liver and are approximately 50% protein. When the particle is secreted by the liver, the core region is relatively empty. HDL is often called "good cholesterol" which finds and removes, stuck LDL at the apo B and E receptors of peripheral cells and brings them back to the liver, where they are excreted. Hepatic uptake of HDLs or reverse cholesterol transport (RCT) may be mediated through an HDL-specific apo A-1 receptor. HDLs also get cholesterol from macrophages by the same way. Moreover, HDL influences the atherogenic process not only through cholesterol extraction but also through anti-inflammatory and antioxidant properties (Fazio and Linton, 2010; Jain *et al*., 2007).

The primary apoprotein of HDL are apo A-I, apo C-I, apo C-II and apo E. Infact, the major function of HDL is to act as circulating stores of apo C-I, apo C-II and apo E (Bhatnagar, 1998; Boes *et al*., 2009).

HDLs are converted into spherical lipoproteins particles through accumulation of CEs. The accumulation converts HDLs to small, dense spherical HDL_3 and large, less dense spherical HDL_2. Any free cholesterol present in CM remnant and VLDL remnants IDLs can be esterified through the action of LCAT (Figure 2). HDLs also acquire cholesterol by extracting it from cell surface membranes, which lead to lowering of intracellular cholesterol, since the cholesterol stored within cells as CE will be mobilized to replace the cholesterol removed from the plasma membrane (Chung and Wasan, 2004).

The cholesteryl esters of HDLs can also be transferred to VLDLs and LDLs through the action of HDL-associated enzyme, cholesterol ester transfer protein (CETP). This has the added effect of allowing the excess cellular cholesterol to be returned to the liver

through the LDL-receptor pathway as well as the HDL-receptor pathway (Brown, 2007).

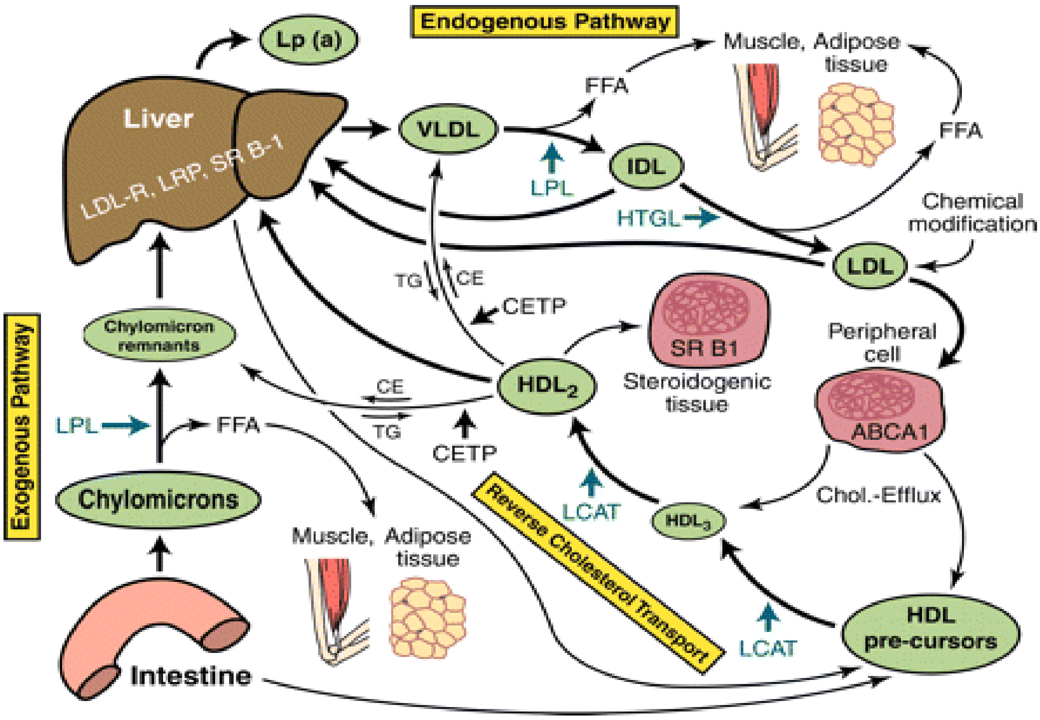


Figure 2. Metabolic pathway of lipoproteins.

1.2. Enzymes modulating lipoprotein metabolism:

1.2.1. Lipoprotein lipase (LPL):

LPL located on endothelial lining of arteries and capillaries and is produced mainly by the adipose, heart, and muscle tissues and to some extent by macrophages. It is a critical enzyme involved in lipolysis of triglyceride-rich lipoproteins, CMs and VLDL. LPL does not act directly on HDL, but its action on triglyceride- rich lipoproteins has an important indirect effect on HDL metabolism (Figure 2). Apo CII is necessary co-factor for its action (Bhatnagar, 1998; Boes *et al*., 2009).

1.2.2. Hepatic triglyceride lipase (HTGL):

It is expressed in the liver and adrenal glands. One of the principal functions of hepatic lipase is to convert IDL to LDL, it specifically catalyzes the hydrolysis of triglycerides, diglycerides and phospholipids in native lipoproteins. It appears to be involved in the selective uptake of CE from HDL (Bhatnagar, 1998; Boes *et al*., 2009).

1.2.3. Lecithin cholesterol acyl transferase (LCAT):

This enzyme is synthesized in the liver and so named because it transfers a fatty acid from the C-2 position of lecithin to the C3–OH of cholesterol, generating a CE and lysolecithin. The esters that are formed move into the core of HDL, enabling the HDL particles to acquire more free cholesterol from other lipoproteins and cell membranes. The activity of LCAT requires interaction with apo A-I, which is found on the surface of HDLs (Bhatnagar, 1998; Brown, 2007).

1.2.4. Cholesterol ester transfer protein (CETP):

Cholesteryl ester transfer protein, a hydrophobic glycoprotein with a molecular weight of 74 kDa, is secreted mainly from the liver and circulates in plasma. Bound mainly to HDL, it transfers CE from HDL to apolipoprotein B (apoB)-containing lipoproteins including VLDLs and LDLs in exchange for TG (Manojit, 2009; Boes *et al*., 2009).

1.2.5. Microsomal triglyceride protein (MTP):

Microsomal triglyceride protein catalyzes the transport of lipid molecules between phospholipid membranes, suggesting that MTP is involved in the synthesis of nascent lipoprotein particles within the lumen and endoplasmic reticulum. It is a key protein in the assembly of apo B containing lipoproteins (Bhatnagar, 1998). The findings that patients with a beta lipoproteinemia have low levels of cholesterol and triglycerides and

that MTP catalyzes lipid transport suggests that interruption of the assembly of apo B containing lipoproteins by inhibition of MTP would lead to a reduction in circulating atherogenic lipid levels (Magnin *et al*., 2003).

1.2.6. Acyl Co-A transferase (ACAT):

An endoplasmic reticulum-bound enzyme which catalyzes intracellular cholesterol esterification, plays important roles in several physiological processes, such as absorption of dietary and biliary cholesterol in the small intestine, secretion of VLDL in the liver, and accumulation of CEs in macrophages in the arterial wall. Cholesteryl esters are stored as cytoplasmic storage droplets or in the lipoprotein secreting cells (Shigehiro *et al*., 2009).

1.3. Regulatory role of cholesterol

Cholesterol is made primarily in the liver (about 1,000 mg per day), but it is also created by cells lining the small intestine and by the individual cells in the body. Cholesterol and other fats cannot dissolve in blood. They have to be transported to and from the cells by special carriers called "lipoproteins" (Bhatnagar, 1998; Rezen *et al*., 2010).

The level of cholesterol regulates several metabolic functions. It suppresses its own synthesis by inhibiting 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase, the rate-limiting enzyme in cholesterol biosynthesis. It activates acyl CoA: cholesterol acyltransferase (ACAT) to form cholesterol within cells. It inhibits synthesis of the LDL receptor. Thus in the absence of exogenous cholesterol, the synthesis of both cholesterol and the LDL receptor are upregulated. These metabolic regulatory effects maintain cellular cholesterol homeostasis. (Figure 3) below summarizes the key steps involved in the biosynthesis of cholesterol (Jain *et al*., 2007).

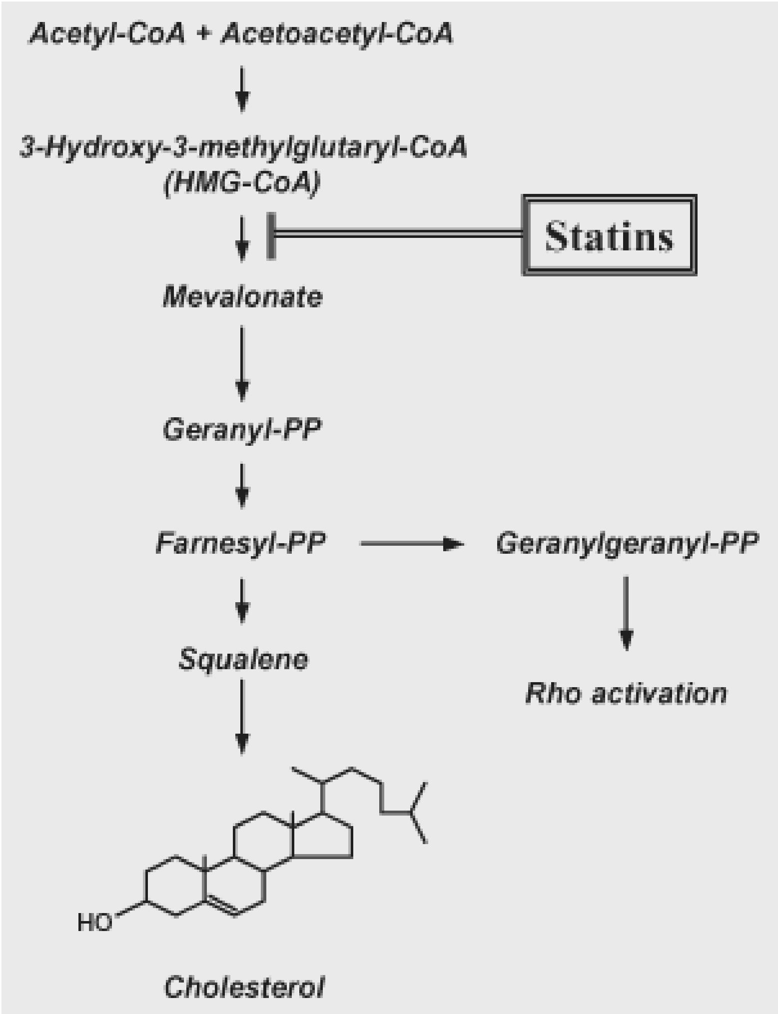


Figure 3. The biosynthetic pathway for cholesterol production in vivo.

1.4. Macrophage foam cell formation:

Macrophage foam cell formation is a critical early event in the pathology of atherosclerosis that is initiated by vessel injury, which allows LDL to penetrate the vessel intimae (Figure 4) (Saha *et al*., 2009). LDL then oxidized by tissue oxidase that is present in vessel intimae to (oxLDL). Inflammatory cells such as macrophages are recruited to the site of injury, recognize and take up oxLDL, which is scavenger receptor-mediated (Blumenthal, 2000). Cholesterol is removed from intracellular

oxLDL and stored in the form of cholesterol esters. Excessive cholesterol ester accumulation causes foam cell formation (fatty streak), releases of pro- inflammatory cytokines, propagation and amplification of inflammation and formation of necrotic core, fibrosis and advancement of lesion formation. Cholesterol can be removed from foam cells by efflux to HDL which is ABCA1 [member 1 of human transporter sub-family ATP-binding cassette transporter (ABCA), also known as the cholesterol efflux regulatory protein (CERP) is a protein which in humans is encoded by the ABCA1 gene] transporter mediated, reverse cholesterol transport, or transport to the liver and disposal by secretion in the bile (as cholesterol or after conversion to bile acids) (Kwiterovich, 1998; Vosper, *et al*., 2002; Davidson and Toth, 2004).

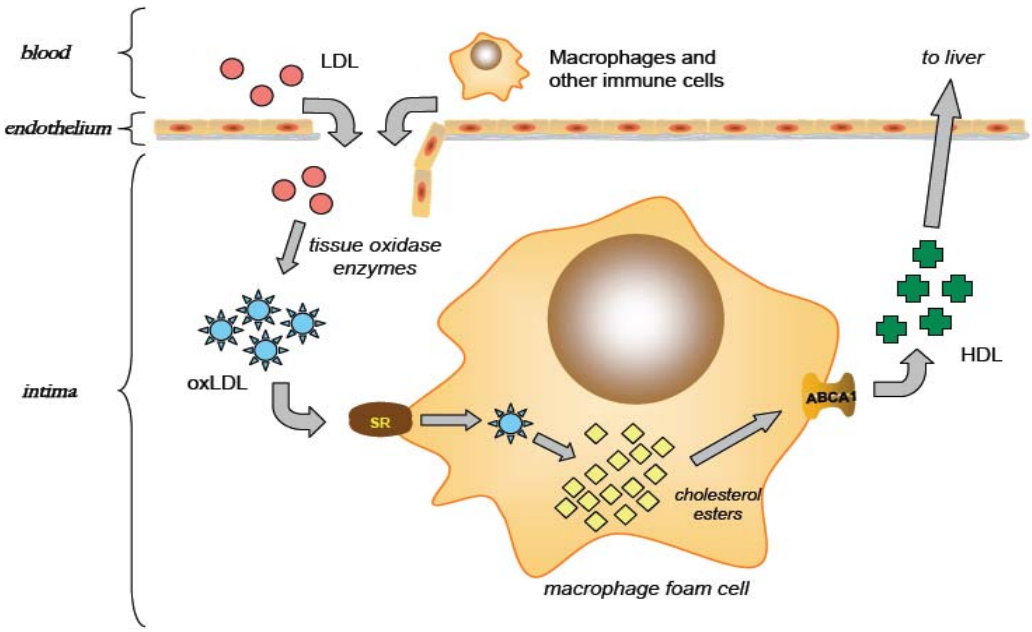


Figure 4. LDL oxidation pathway (macrophage foam cell formation)

2. Classification of antihyperlipidemic drugs:

Several different classes of drugs are used to treat hyperlipidemia. These classes differ not only in their mechanism of action but also in the type of lipid reduction and the magnitude of the reduction. Statins, the most common group of antihyperlipidemic drugs lower cholesterol by interrupting the cholesterol biosynthetic pathway (Yoshihisa and Yuichi, 2006). On the other hand, fibrate group decrease fatty acid and triglyceride levels by stimulating the peroxisomal β-oxidation pathway (Jun *et al*., 2010). Apart from these drugs, ezetimibe, selectively inhibits intestinal cholesterol absorption (Davidson and Toth, 2004), cholestyramine, colestipol, and colesevelam, sequester bile acids (Guyton and Goldberg, 2009), torcetrapib, inhibits cholesterol ester transfer protein (Neeli and Rader, 2008), avasimibe, inhibits acyl-CoA cholesterol acyltransferase (Burnett *et al*., 2005), implitapide, inhibits microsomal triglyceride transfer protein (Li *et al*., 2006), and niacin, modifies lipoproteins (Chapman *et al*., 2010), are several options to treat hyperlipidemia. However, statins and fibrates are the most popular in terms of medical use and importance (Kwang *et al*., 2008).

2.1. Hydroxymethyl glutarate coenzyme A (HMG-CoA) reductase inhibitors (statins):

The statins work by competitively inhibiting 3-hydroxy-3-methylglutaryl CoA (HMG-CoA) reductase, the rate limiting enzyme involved in the biosynthesis of cholesterol (Mikio *et al*., 2001; Valeriy *et al*. 2008). When cholesterol biosynthesis is inhibited, the low density lipoprotein cholesterol (LDL-C) receptor is upregulated and LDL-C is rapidly cleared from the bloodstream. In addition, statins have been shown to lower very low density lipoprotein cholesterol (VLDL-C) and triglycerides, and sometimes

raise HDL-C but to a lower extent than lowering LDL-C (Dergunov *et al*., 2008; Farnier and Davignon, 1998).

Statins decrease the concentration of all apo B containing lipoproteins by reducing the production of VLDL, as a result of cholesterol synthesis inhibition, and by upregulating LDL receptors, which also remove VLDL remnants (IDL) from the circulation (Ballantyne *et al*., 2008).

2.1.1. Structure activity relationships:

The structure of statins is characterized by desmethylmevalonic acid or the lactone, the pharmacophore which is connected to a lipophilic ring, such as hexahydronaphthalene, indole, pyrrole (Pfefferkorn *et al*., 2007), pyrimidine, or quinoline, by a linking element (a two-carbon spacer) (Figure 5) (Mikio *et al*., 2001; Zhengyan *et al*., 2007).

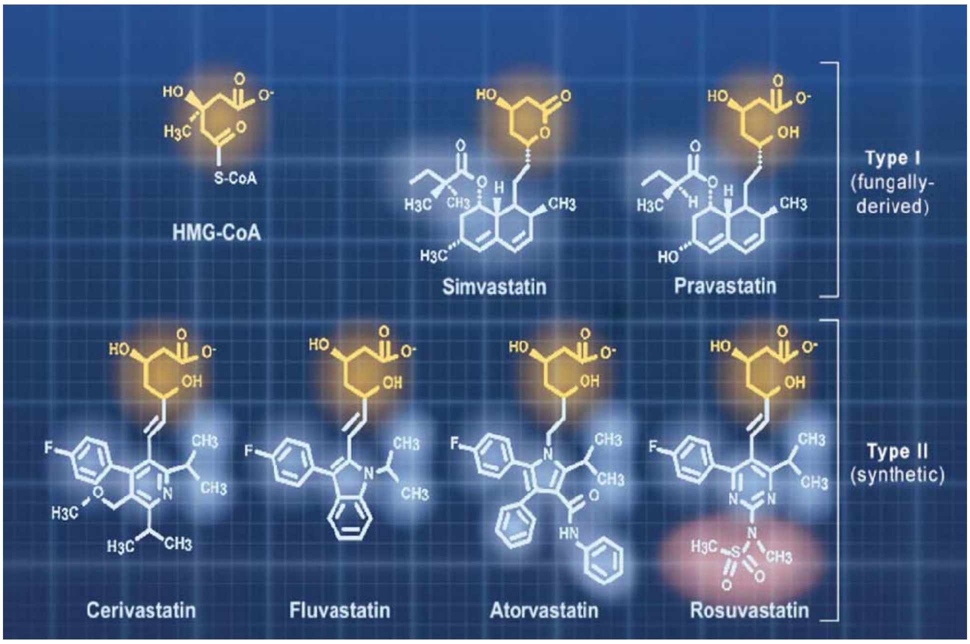
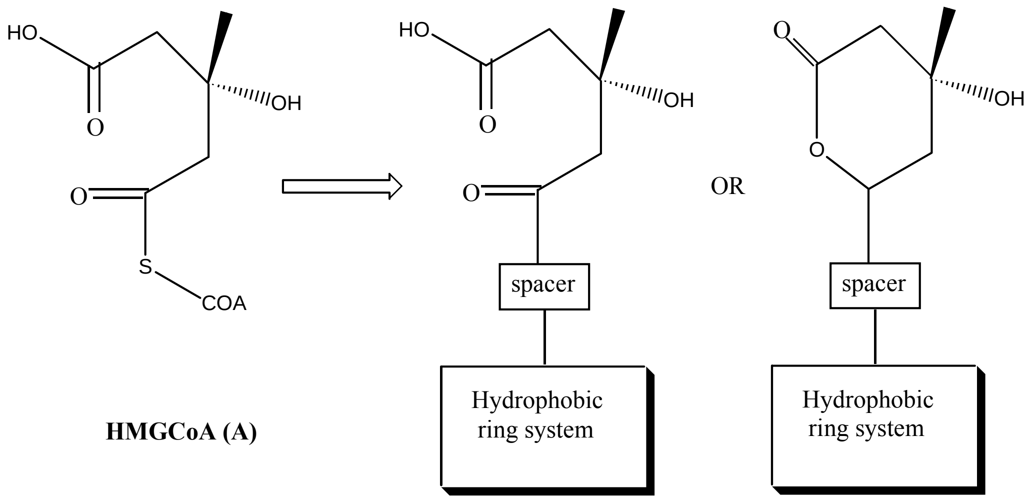


Figure 5. Two-dimensional chemical structures of statin

All of the statins mimic the substrate HMGCoA (A) for the HMG-CoA reductase enzyme. The general structure of the inhibitors contains a central hydrophobic ring system, with a hydroxy-acid (B) or lactone (C; hydroxyacid prodrug, which is

hydrolyzed to the active open acid form in vivo) side-chain necessary for anchoring into the active site of the enzyme.



HMGCoA (A) **(B)** **(C)**

The tight binding of the inhibitors into the enzyme blocks access of the substrate, HMG-CoA (A), to the active site, although the exact binding mode was unknown for some 25 years. Recently, the co-crystallization of several statins bound in the catalytic portion of the human enzyme has permitted the elucidation of the binding mode of the inhibitors. The statins exploit the conformational flexibility of the enzyme, to create a hydrophobic binding pocket near the active site for the hydrophobic ring system of the inhibitor, thus occupying both the HMG-binding pocket and a part of the binding surface for the CoA, see (Figure 6) below (Roche, 2005).

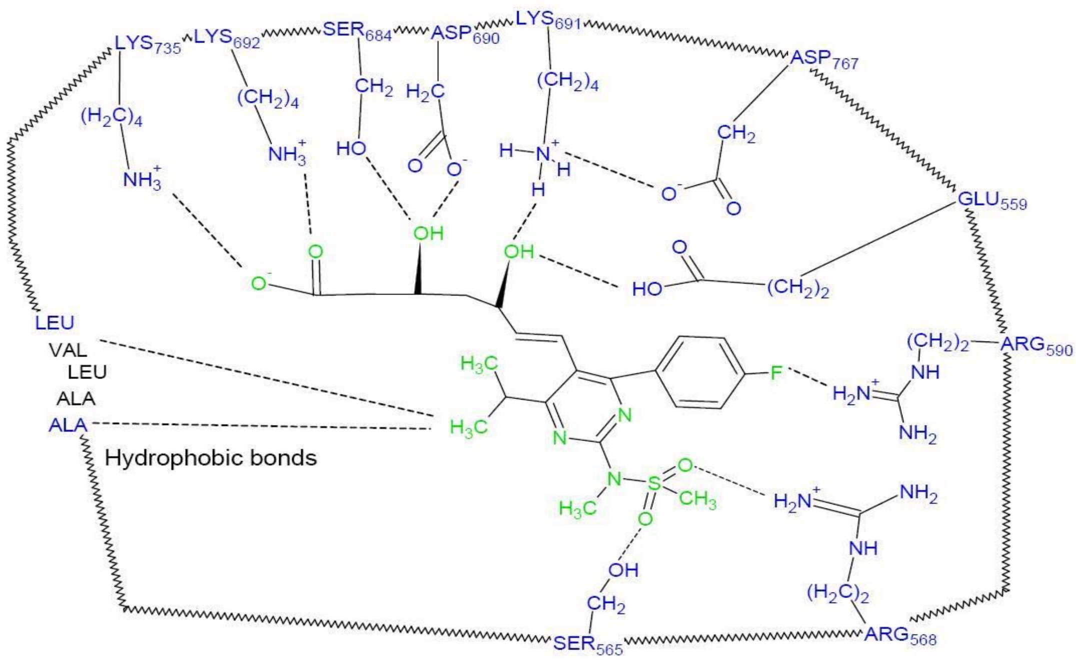


Figure 6. Co-crystallization of rosuvastatin bound in the catalytic portion of the human enzyme.

Statins can be classified into naturally derived and chemically synthesized (Farnier and Davignon, 1998). The first statin identified was Mevastatin, which is not in use now (Akira, 2004). Cerivastatin was withdrawn from the market by its manufacturers in 2001 after reports of rhabdomyolysis on account of over 100 fatal cases world-wide (Bakri *et al*., 2003). Pitavastatin is a new statin available in Japan that might affect cerebral arteries directly to improve cerebral blood flow (Yoshihiko *et al*., 2009).

Lovastatin and simvastatin are prodrugs that are converted into their active forms in the liver, whereas the other statins are active in their parent forms (Davidson and Toth, 2004). All statins show similar function by binding to the active site of HMG-CoA reductase and in this way inhibit the enzyme. However, structural differences in statins are responsible for differences in potency of enzyme inhibition (Corsini *et al*., 2005). All statins differ from HMG-CoA in being more bulky and more hydrophobic. The naturally derived statins " lovastatin, pravastatin, and simvastatin" are modified fungal extracts, contain a substituted decalin ring structure (Farnier and Davignon, 1998).

Fully synthetic statins "fluvastatin, cerivastatin, atorvastatin, (p)itavastatin (NK-104), and rosuvastatin" with larger flurophenyl groups are linked to the HMG like moiety. These additional groups change the character from very hydrophobic to partly hydrophobic. The most lipophilic compounds are simvastatin and lovastatin, while pravastatin and rosuvastatin are the most hydrophilic (Figure 7) (Davidson and Toth, 2004).

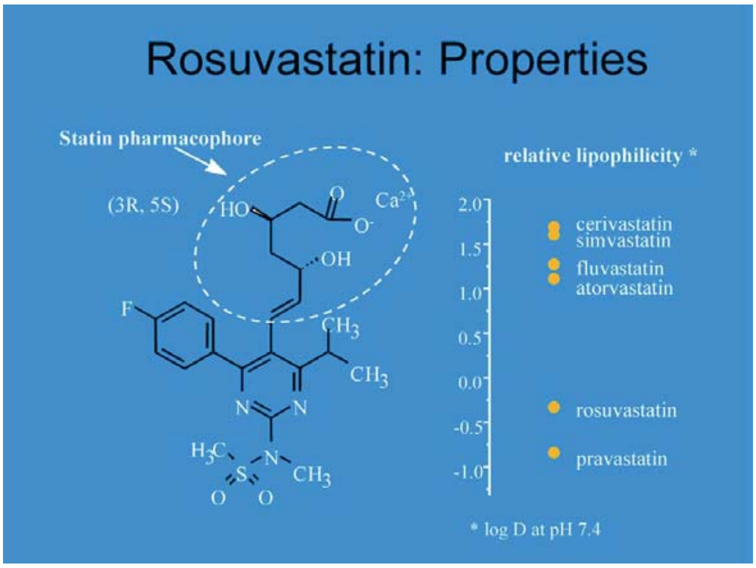


Figure 7. Chemical structure of rosuvastatin and the relative lipophilicity of the various statins.

The absorption of statins varies from 30 % to 98 %. All statins are rapidly absorbed after oral administration and achieve the peak concentrations level within 4 hours. Food has no effect on bioavailability of statins, except for lovastatin where it is increased. Statins have a slow onset of effect and are, therefore insensitive to temporary changes in unbound plasma drug concentration. The statins have a variety of excretion pathways. Furthermore, statins reduce the susceptibility of lipoproteins to oxidation, both *in vitro* and *ex vivo*. Oxidative modification of LDL appears to play a key role in mediating the uptake of lipoprotein cholesterol by macrophages. Results from multiple clinical studies have indicated that statins may be beneficial for restoring endothelial function,

stimulating bone formation, decreasing vascular inflammation (Wierzbicki *et al*., 2003), prevention of diabetes mellitus (DM), diabetic retinopathy, age related macular degeneration, and diabetic and non diabetic nephropathy (Baghdasarian *et al*., 2004) and enhancing the stability of plaques associated with atherosclerosis (Rauch *et al*., 2000).

2.1.2. Adverse effects of statin therapy

The principal side effects of statins are gastrointestinal disturbance (nausea, bloating, diarrhoea, and constipation), abnormal liver transaminases, and myalgia myositis and in different clinical studies occur in 0.1–5% of patients (Wierzbicki *et al*., 2003; Schmitz and Langmann, 2006). In rare cases, statins can cause rhabdomyolysis and myositis (painful myalgia with a significant creatine kinase release [>2000 IU/L]). Other adverse effects are: hepatic dysfunction, renal insufficiency, hypothyroidism and serious infections. Case reports of demyelinating neuropathies caused by statins suggest that, in some individuals, the actions of statins in reducing glycosphingolipid synthesis may be significant (Jeppesen *et al*., 1999). In an effort to reduce the potential for myalgia, there has been increased emphasis on the design of more hepatoselective HMG-CoA reductase inhibitors (Antonios and Christie, 2002; Larsen *et al*., 2007).

2.2. Bile acid sequestrants (BAS):

Bile acid sequestrants have long been used for serum cholesterol reduction. The basic mechanism of action of the sequestrant is to bind anionic metabolites, present in the enterohepatic circulation, to the insoluble, positively charged polymer, and thus make them unavailable for further recirculation or micelle formation. The binding of soluble anions to the anion-exchange polymers is believed to take place by two mechanisms ionic and non-ionic bindings. Therefore the polarity of the polymer skeleton, as well as

the number and nature of the cationic groups, should be of importance in determining the strength of binding of soluble anions to the sequestrants. The liver must, therefore, produce additional bile salts by enzymatically cleaving cholesterol. Some of this cholesterol is derived from the plasma, resulting in a net reduction in plasma levels of cholesterol (Schreiber *et al*., 2001).

Consequently the liver's pool of bile acids depletes, leading to increased conversion of cholesterol to bile acid in hepatocytes. Decline in hepatic cholesterol content stimulates the production of LDL receptors and also increases synthesis of cholesterol in the liver. The increase in hepatic LDL receptors increases LDL clearance and lowers LDL-C levels. However this effect is partially offset by the enhanced cholesterol synthesis caused by the upregulation of HMG-CoA reductase. This leads to the increased hepatic conversion of cholesterol to bile acid through upregulation of cholesterol 7α-hydroxylase activity. Bile acid sequestrants have a very slight effect on HDL-C and can lead to TG elevations (Gotto and DPhil, 2002).

Bile acid sequestrants (anion-exchange resins) are highly positively charged resins and bind to the negatively charged bile acids. Because of their large size, these resins are not absorbed and thus the bound bile acids are excreted in the stool and therefore, have minimal systemic side effects. Commercially available bile acid sequestrants, cholestyramine (Figure 8), colesevalam and colestipol, are polymers. They bind bile acids by a non-specific ion exchange mechanism. Due to the non-specific binding of bile acids to these compounds, relatively large doses of each are required for an effective reduction in plasma cholesterol levels (Parkinson *et al*., 1970).

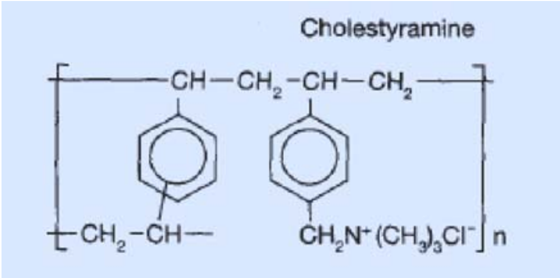


Figure 8. Molecular structure of bile acid sequestrants, cholestyramine.

2.2.1. Adverse effects of BAS therapy:

An adverse side effect associated with BAS include unpleasant sandy and gritty taste causing nausea, abdominal discomfort, indigestion, impaction, aggravation of hemorrhoids and constipation may occur.

Due to the increase of TG concentrations in the blood plasma, BAS drugs often transiently increase the activities of alkaline phosphatase and hepatitic transaminases and hyperchloraemic acidosis can occur as a result of these drugs being in the chloride form of an anion exchange resin. Increasing the dosages to higher than recommended has resulted in steatorrhea, in which fat-soluble vitamin absorption is severely impaired and vitamin supplements are usually recommended for such side effects (Schmitz and Langmann, 2006; Zarras and Vogl, 1999). Because of these drawbacks, BAS are generally regarded as second line or additive agents for treating hypercholesterolemia. They can be drugs of choice in situations in which patient safety is the key consideration, such as in children or in women of childbearing potential (Alfons *et al*., 1998).

2.3. Cholesterol absorption inhibitors:

Cholesterol absorption inhibitor functions by decreasing the absorption of cholesterol in the small intestine. This cause a decrease in the cholesterol delivery to the liver which

in turn clears more cholesterol from the blood. Ezetimibe (Figure 9) is the first of this class of drugs.

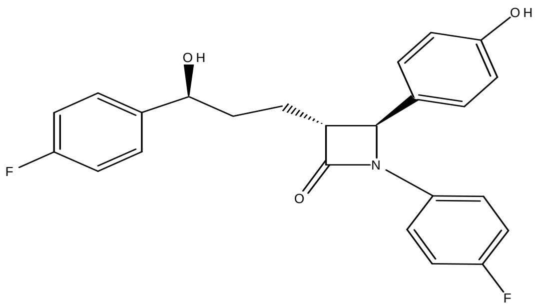


Figure 9. Molecular structure of Ezetimibe

Ezetimibe stays at the brush border of the small intestine and selectively inhibits the absorption of cholesterol from the intestinal lumen into enterocytes. It interacts with the Niemann Pick C1-like 1 (NPC1L1) protein which plays a critical role in cholesterol transporting through the intestine wall (Xianxiu *et al.*, 2007). Thus NPC1L1 lies in the ezetimibe sensitive pathway for cholesterol absorption, making it a likely candidate for the target of ezetimibe (Pfefferkorn *et al.*, 2008; Yubin *et al.*, 2009).

After oral administration, ezetimibe is glucuronidated rapidly in the intestines, and once it is glucuronidated, undergoes enterohepatic recirculation, and hence deliver the drug repeatedly to its site of action. The glucuronide of ezetimibe is much more effective than the parent drug, mainly because of its localization at the brush border of the intestines. Ezetimibe does not interact with drugs metabolized by CYP 1A2, 2D6, 2C8, 2C9, or 3A4, suggesting that it has low potential for participating in drug-drug interactions. Importantly, ezetimibe does not interact with statins. Accordingly, ezetimibe can be taken at the same time as the statin during coadministration therapy.

Monotherapy with ezetimibe can effectively reduce LDL cholesterol in patients having hypercholesterolemia. Ezetimibe does not affect absorption of triglycerides, fatty acids, bile acids, or fat-soluble vitamins, including vitamins A, D, E, and β carotenes (Davidson and Toth, 2004).

Ezetimibe reduces the delivery of dietary and biliary cholesterol from the intestine to the liver, thus decreasing hepatic cholesterol stores and up-regulating hepatic (HMG)-CoA reductase activity. Its inhibition of cholesterol absorption therefore complements the decrease in cholesterol synthesis induced by statins. Combination therapy with statins and ezetimibe that act via a complementary pathway may allow additional patients to achieve their recommended cholesterol goals (Shinichiro and Keijiro, 2008).

2.3.1. Structure activity relationships:

The key structural elements identified for cholesterol absorption inhibition were (Yubin *et al*., 2009):

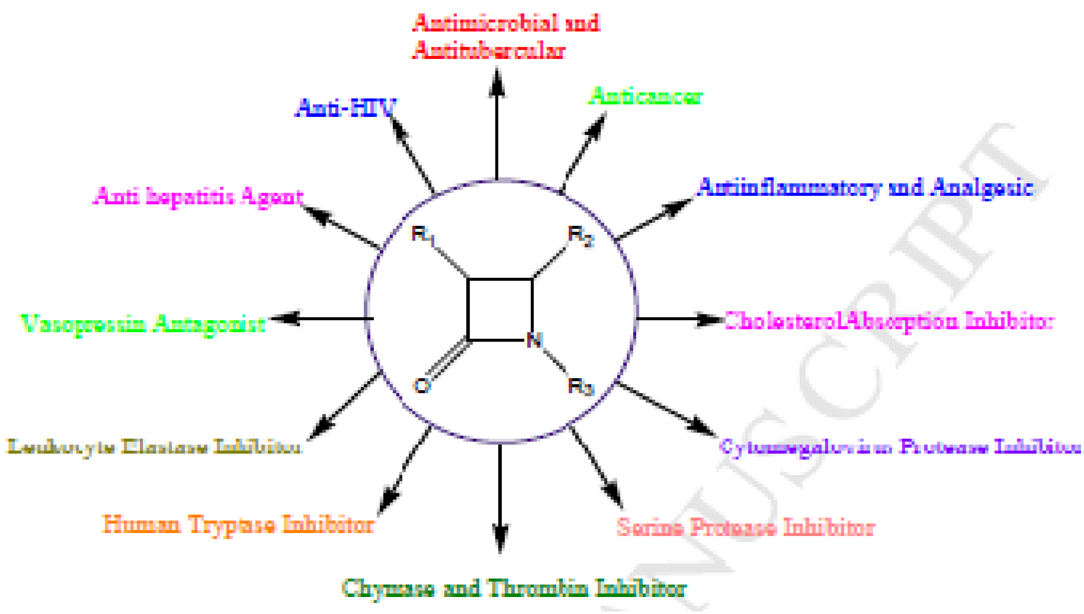
(1) Azetidinone as the required backbone (Mehta *et al*., 2010);

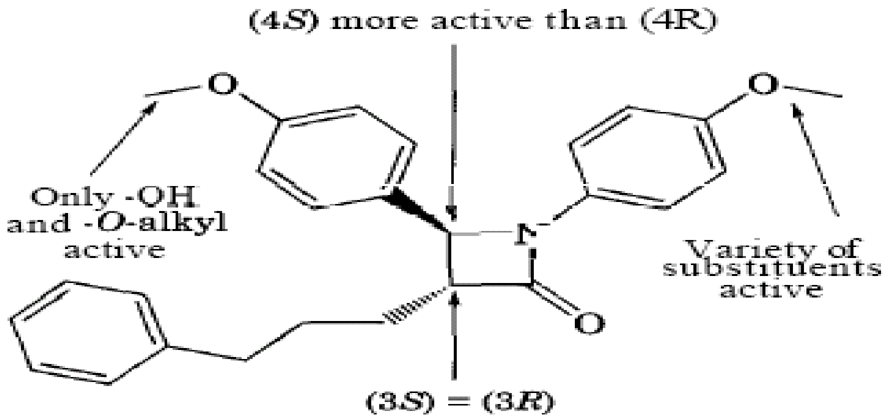
(2) N-phenyl is required, but could tolerate a range of substituents;

(3) The phenyl tethered with three-carbon chain at 3-position is necessary for activity;

(4) p-hydroxyl or p-methoxyphenyl at 4-position is a necessity;

(5) unlike the chiral center at C-4 which shows a clear preference for S configuration both 3S and 3R forms often demonstrate comparable activity without consistent preference (Xianxiu *et al*., 2007).





(40)

2.3.2. Adverse effects of Ezetimibe:

The adverse effects of ezetimibe are few and mild. In most studies ezetimibe does not increase myopathy or rhabdomyolysis, whether used alone or in combination with statins, although some case reports of myopathy were there due to this agent. In addition, ezetimibe can cause mild elevations of liver transaminases, when used in combination with a statin. Other side effects are extremely rare (Schmitz and Langmann, 2006). The most commonly reported adverse effects are upper respiratory

tract infection, diarrhea, headache, arthralgias, back pain, musculoskeletal pain, and viral infections (Jeu and Cheng, 2003).

2.4. Niacin:

Niacin, a drug in use since the 1950s (Figure 10) a water-soluble vitamin of type B (nicotinic acid), functions in the body after conversion to nicotinamide adenine dinucleotide (NAD) in the NAD coenzyme system. Niacin lowers VLDL-C, LDL-C, and TG, and increases HDL-C (Boatman *et al*., 2010). Niacin inhibits the lipolysis of triglycerides by hormone-sensitive lipase, which reduces transport of free fatty acids to liver and decreases hepatic triglyceride synthesis. In liver niacin reduces TG synthesis by inhibiting both the synthesis and esterification of fatty acids that increases apo B degradation (Jain, *et al*., 2007).

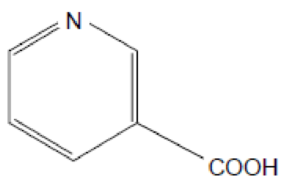


Figure 10. Molecular structure of niacin

Reduction of TG synthesis reduces hepatic VLDL production, which accounts for the reduced LDL levels. Niacin also enhances LPL activity, which promotes the clearance of CMs and VLDL triglycerides. Niacin shows the most dramatic HDL elevation (20–35%) by decreasing the fractional clearance of apo A-1 in HDL, rather than by enhancing HDL synthesis, but its use has been limited by side effects.

Early efforts to improve upon niacin met with little success because its mechanism of action was not known. Recent renewed interest in HDL raising drugs spurred efforts to identify niacin's cellular target, a G-protein-coupled orphan membrane receptor,

GPR109A, also referred to as HM74A or 19CD; this receptor is highly expressed in adipose tissue but equally in cells of the immune system, including monocytes and macrophages. In vitro, this receptor showed a high affinity for niacin (Schmidt *et al*., 2010).

2.4.1. Adverse effects of niacin:

Patients on niacin therapy experience intense facial reddening called flushing (i.e., warmth, redness, itching and/or tingling), which causes low patient compliance with treatment. Additionally, glucose tolerance is reduced in patients taking niacin, limiting its use in diabetic and prediabetic patients. Hyperuricemia or gout and GI distress is encountered too. Liver toxicity can be a problem as well, especially in sustained release formula (Schmidt *et al*., 2010).

Recently, a new preparation of niacin has been developed that is differentiated by its association with the antiflushing agent laropiprant, produced marked and durable beneficial changes in lipid and lipoprotein parameters in patients with primary hypercholesterolemia or mixed dyslipidemia in association with improved niacin tolerability with respect to flushing (Chapman *et al*., 2010).

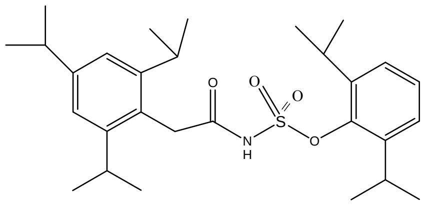
2.5. Acyl-CoA cholesterol acyl transferase inhibitors (ACATI):

In mammalian cells, cholesterol is the predominant cellular sterol, and cholesterol esterification is catalyzed by the enzyme ACAT. ACAT activity has been implicated in a number of physiologic processes. In the small intestine, ACAT has been proposed to play a role in cholesterol absorption by maintaining a free cholesterol diffusion gradient across the enterocyte surface through the formation of cholesterol esters intracellularly. Cholesterol ester formation by ACAT also has been hypothesized to be important for the assembly and secretion of apo-B containing lipoproteins in the intestine and the

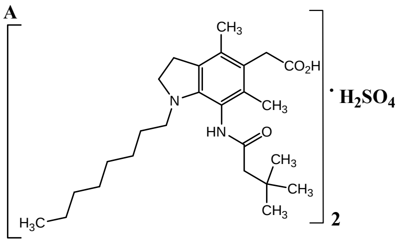
liver. In the adrenal glands and other steroidogenic tissues, ACAT synthesizes cholesterol esters that accumulate in cytosolic droplets where they can serve as cholesterol substrate stores for steroidogenesis. In macrophages, ACAT generates intracellular cholesterol esters that are stored as cytosolic lipid droplets, a characteristic feature of macrophage foam cells in atherosclerotic lesions (Natarajan *et al*., 2010).

Two isoforms of ACAT have been identified (ACAT-1 and ACAT-2). ACAT-1 is present in many cell types, including macrophages, and ACAT-2 is active in the intestine and liver. In these intestinal cells, it promotes incorporation of dietary cholesterol into CMs for transport to the liver. In hepatocytes, esterification of free cholesterol precedes its incorporation into VLDL particles. In theory, inhibition of ACAT-1 could prevent the transformation of macrophages into foam cells in the vessel wall. Subsequently, free cholesterol would be available for reverse cholesterol transport. In addition, inhibition of ACAT-2 could decrease serum lipid levels by reducing the synthesis of lipoproteins and would reduce LDL formation (Meuwese *et al*., 2009; Natarajan *et al*., 2010).

A number of ACAT inhibitors have been developed for use as anti-atherosclerosis agents. Some of these act by inhibition of intestinal cholesterol absorption or hepatic lipoprotein synthesis and secretion , and others, as specific inhibitors of macrophage foam cell formation. Avasimibe a nonselective ACAT inhibitor (Burnett *et al*., 2005), *in-vitro* studies of human macrophages, have demonstrated that it reduces foam cell formation not only by enhancing free cholesterol efflux, but by inhibiting the uptake of modified LDL. Avasimibe decreases apo-B secretion; an effect associated with increased intracellular degradation. Avasimibe stimulates bile acid synthesis in primary rat hepatocytes by increasing the supply of free cholesterol both as substrate and by induction of cholesterol 7α-hydroxylase (Norata and Catapano, 2004).



Avasimibe



Pactimibe

Pactimibe (CS-505) is a potent inhibitor of both ACAT-1 and ACAT-2. Treatment with ACATIs showed promising results for the prevention of atherosclerosis in various animal models (Terasaka *et al*., 2007).

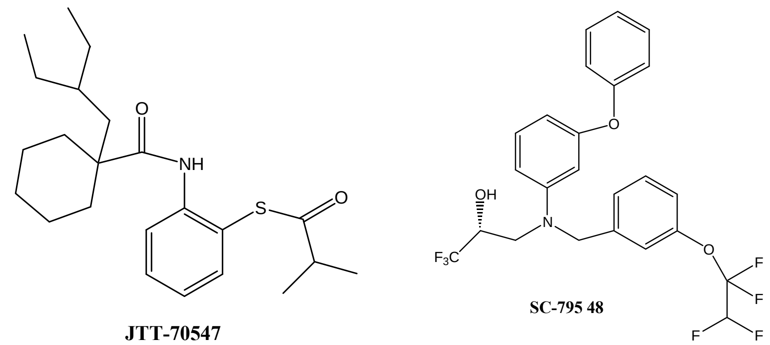
In contrast to promising results in experimental animal models, all subsequent clinical studies in human with ACATI failed to show lipid profile changes, as well as, reductions surrogate markers for coronary artery diseases. Infact there was even a tendency to be a proatherogenic. Potential explanations for this proatherogenic effect of ACAT inhibition is that the accumulation of free cholesterol in macrophages results in ABCA1 degradation, limiting RCT, and such free cholesterol abundance causes cellular death, stimulating and intensifying the development of atherosclerosis (Meuwese *et al*., 2009; Natarajan *et al*., 2010).

2.6. Cholesteryl ester transfer protein (CETP) inhibitors:

CETP is a hydrophobic glycoprotein that is secreted mainly from the liver and it circulates in plasma, bound mainly to HDL. CETP promotes the transfer of cholesteryl ester in the plasma from the HDL lipoprotein fraction to the potentially proatherogenic VLDL and LDL lipoprotein fractions, as well as the transfer of TG from the latter to the HDL lipoproteins. Thus, CETP-mediated transfers from HDL to VLDL and LDL provide a potential indirect pathway by which HDL cholesteryl esters can be delivered to the liver (Marcelo *et al*., 2007).

However, there is also evidence that CETP may be involved in RCT. Most experimental evidences in animals favor a proatherogenic role for CETP and support a view that inhibition of CETP is antiatherogenic. Inhibitors of CETP have the potential to inhibit atherogenesis by enhancing the rate of RCT (Jain, *et al*., 2007).

Thus, CETP offers a model target for raising HDL and inhibiting atherosclerosis. JTT-705 47 (dalcetrapib) is a compound that inhibits CETP activity by forming a disulfide bond with protein. A trifluoro-3-amino-2-propanol (SC-795) 48 was also reported (Figure 11) (Manojit, 2009).



JTT-70547

SC-795 48

Figure 11. Molecular structure of JTT-705 and SC-795.

Genetic deficiency and genetic variants of CETP that lead to lower CETP activity are associated with elevated HDL-C levels and reduced risk of CVD as well, although this point has been controversial. These data have prompted evaluation of the therapeutic potential of CETP inhibitors as HDL-raising agents in reducing vascular risk. However, the first of the developmental CETP inhibitors, torcetrapib, (Figure 12) has been shown to be a flawed drug. Despite substantial elevation in HDL cholesterol levels (by 72%), however, it was associated with an excess of deaths and CV events, leading to the early discontinuation of the torcetrapib clinical development program. Whereas the precise reasons for the failure of torcetrapib remain elusive, treatment with this agent was

associated with an increase in blood pressure, alterations in serum electrolytes, and an increase in circulating aldosterone levels (Cannon *et al*., 2009).

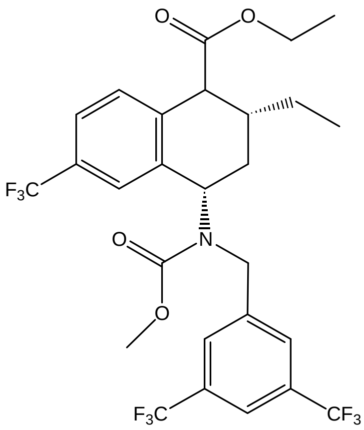


Figure 12. Molecular structure of torcetrapib.

These results led to the early termination of the trial and of torcetrapib development. Imaging studies also failed to show any benefit of torcetrapib (plus atorvastatin) on atherosclerosis progression in patients with clinical evidence of CAD, familial hypercholesterolemia, or mixed dyslipidemia, despite substantial increases in levels of HDL-C and decreases in LDL-C. While the discovery of torcetrapib was expected to open up a new category in the field of cardioprevention, the failure of its clinical trial has now raised several questions on the role of LDL-C and HDL-C on atherosclerosis and the future of CETP inhibitors as therapeutic drugs. Moreover, it is still not clear whether the increased risk of mortality observed in patients treated with torcetrapib during the clinical trials was due to an increase in blood pressure or to the some other unknown 'off-target' effect. Currently there are no marketed CETP inhibitors, although there are several compounds currently in clinical trials (Figure 13) (Rano *et al*., 2009).

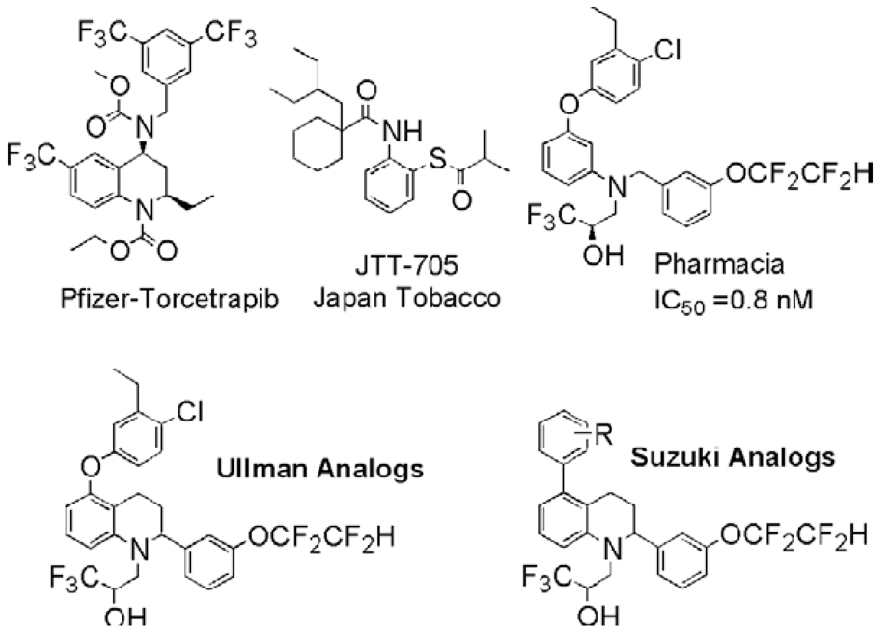


Figure 13. Structures of current CETP inhibitors.

2.7. Fibrates or lipoprotein lipase stimulants (fibric acid derivatives):

Fibrates were first introduced in clinical trials in the early 1970s to assess their role in cardiovascular prevention. Fibrates have been studied for more than 40 years and are clearly effective at raising HDL cholesterol, lowering TG concentrations, and could also reduce LDL-C and CM remnants, and hence were thought to be particularly useful in patients with non LDL dyslipidemia characterized by high TG and VLDL levels and low HDL-C levels (Saha *et al*., 2007; Abourbih *et al*., 2009; Jun *et al*., 2010).

The fibrates (Figure 14) affect lipid metabolism as agonists of the enzyme peroxisome proliferator activated receptor alpha (PPARα) an important family of nuclear receptors involved in the transcription of genes involved in fatty acid oxidation, apolipoprotein production, and cholesterol transport, with additional functions in inflammation, endothelial function, and vascular remodeling. This results in the peripheral lipolysis of TG rich lipoproteins through the stimulation of LPL, a reduction in apoprotein C-III, and an increase in apoprotein A-I production. Fibrate mediated increase in HDL-C is

due to PPARα stimulation of apo A-I and apo A-II expression, which increases HDL levels (Jain, *et al*., 2007).

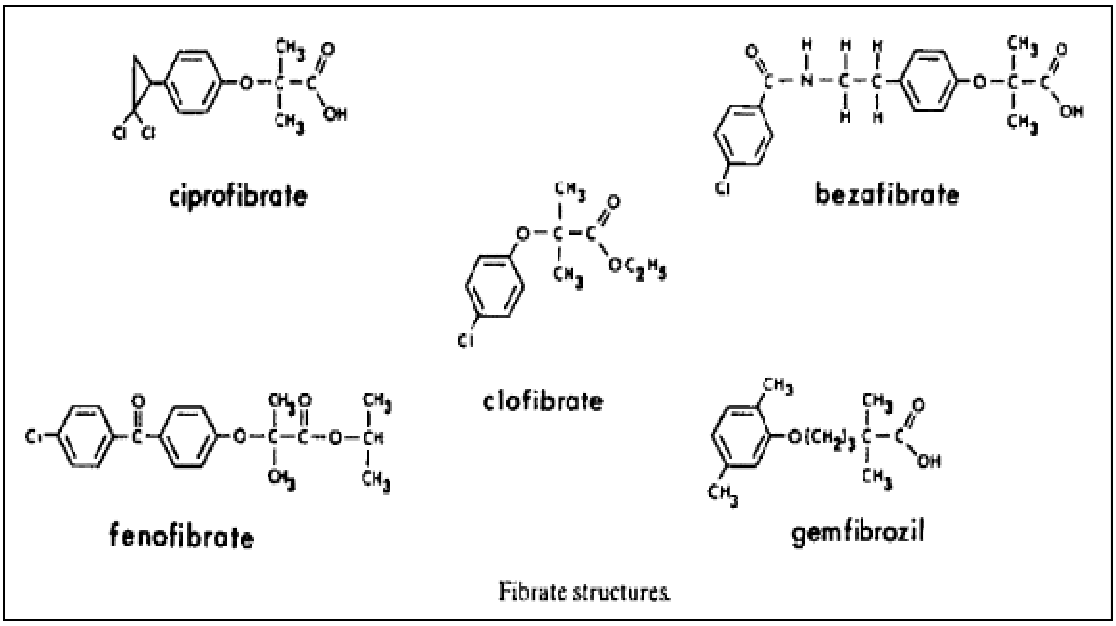
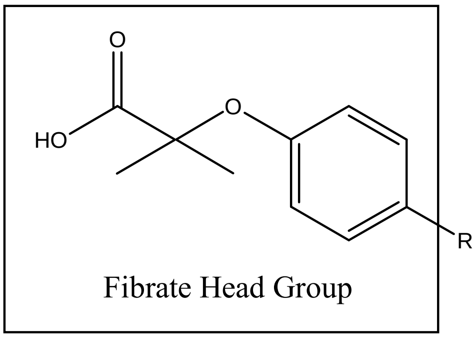


Figure 14. Chemical formulae of principle fibrates.

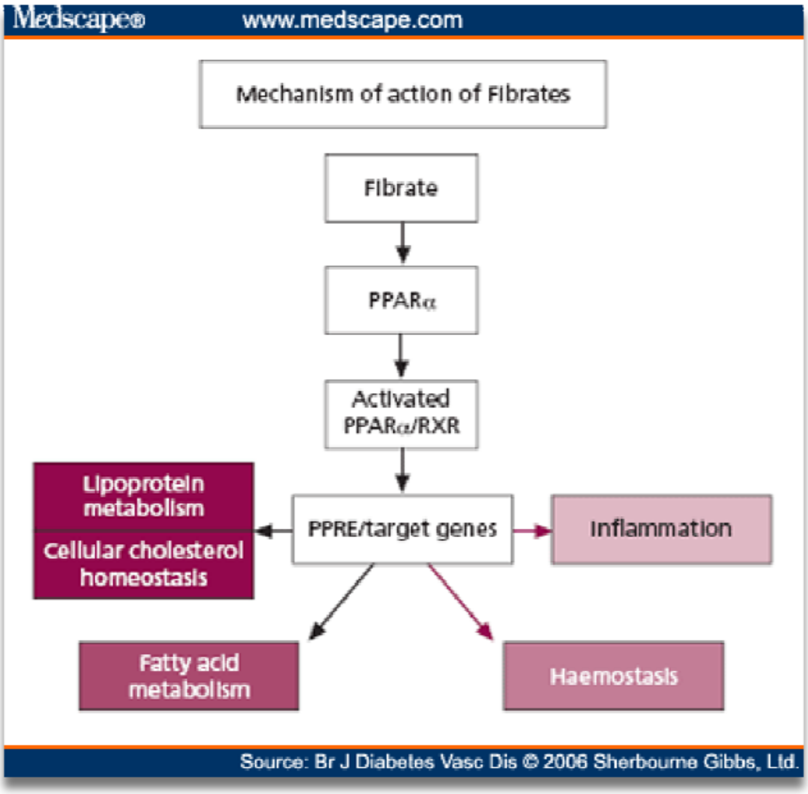
2.7.1. Structure activity relationships:

The primary structural factor of the fibrates necessary for receptor recognition is the "fibrate head group", which is found in all of the synthetic ligands.



2.7.2. Mechanisms of action of fibrates:

The fibrates' primary mode of action is to activate one of a group of nuclear receptors PPARs, specifically PPAR-α. Activation of PPAR-α modulates the expression of several genes involved in lipoprotein metabolism.



The Major mechanisms underlying modulation of lipoprotein phenotypes by fibrates summarized as the following:

1. The activity of LPL is increased and results in an increase in the clearance of circulating triglyceride-rich lipoproteins.

2. The synthesis of apo C-III is decreased. Apo C-III inhibits LPL. Hence, low apo C-III levels will further enhance the clearance of triglyceride-rich lipoproteins. Apo C-III synthesis is also increased in hypertriglyceridemic individuals. Thus reducing apo C-III

gene expression by PPAR-α agonists may enhance both the clearance of the triglyceride-rich lipoproteins and the decrease in their production.

3. PPAR-α agonists also increase hepatic fatty acid oxidation thereby reducing the esterification of free fatty acids and leading to a further decrease in VLDL triglyceride production.

4. Recently, PPAR-α agonists have also been found to increase apo A-V gene expression, the over expression of which results in triglyceride reduction.

5. The fibrates increase HDL production by transcriptional induction of the synthesis of the major HDL apolipoproteins, apo AI and apo A-II, and also enhance RCT through an increase in the adenosine triphosphate binding cassette-1 (ABC-A1) cholesterol transporter and in the scavenger receptor SR-BI/CLA-1 (Shepherd, 1993; Bart *et al.*, 1998; Steiner, 2005).

2.7.3. Adverse effects of fibrate therapy:

Fibrates are generally well tolerated with very few side-effects. The most common side effects include headaches, skin rashes, nausea and gastrointestinal problems. Uncommon side effects include fever, chest pain, reduced libido, breathing difficulty, dizziness, irregular heartbeat and swollen feet or legs. Very rarely, muscular aches and pains or slight liver damage might occur (Virgil, 2007). Fibrates have been described as myopathy inducing agents, but rhabdomyolysis has been seen only during statin-fibrate combination therapy (Hodel, 2002). Fibrates have been known to cause gallstones. Therefore, are contraindicated in hepatic or severe liver dysfunction and previous gallstone disease (Jain, *et al.*, 2007).

2.7.4. Peroxisome proliferation activated receptor (PPAR) agonists:

Peroxisome proliferation activated receptors are a subfamily of the 48-member nuclear receptor super family and regulate gene expression in response to ligand binding. PPARs are ligand-activated transcription factors that bind to specific DNA sequences known as peroxisome proliferator response elements (PPREs) located in the promoters of target genes. Binding of ligand allows for a conformational change in the protein, which results in the binding of co-activator molecules, such as PPAR-binding protein, or PPAR-γ co-activator-1, providing a link with the basic transcriptional machinery and resulting in increased transcription of the target gene, that have been implicated in the regulation of lipids and glucose metabolism, morphogenesis, cell growth, differentiation, and homeostasis (Vosper, *et al*., 2002).

Transactivation effects require ligand-activated dimerisation of PPAR with the retinoid X receptor (RXR, NR2B), followed by translocation of the PPAR : RXR heterodimer complex to the nucleus, whereupon it binds to PPAR response elements of target genes and induces their expression. Transrepression effects are mediated via interference with transcription factors such as activator protein-1 (AP-1) and nuclear factor-κB (NF-κB) (Figure 15). There is evidence that all PPAR ligands do not stimulate transactivation and transrepression pathways to a similar extent, meaning that different agents of the same class may have potentially disparate effects (Feige *et al*., 2006).

Three mammalian PPARs have been identified and are termed as PPAR-α, -γ, and -δ. These share similar structural organization and sequence homology. However, these isoforms possess distinct functions, and vary in their ligand affinity, expression, and activity in different metabolic pathways (Vosper, *et al*., 2002).

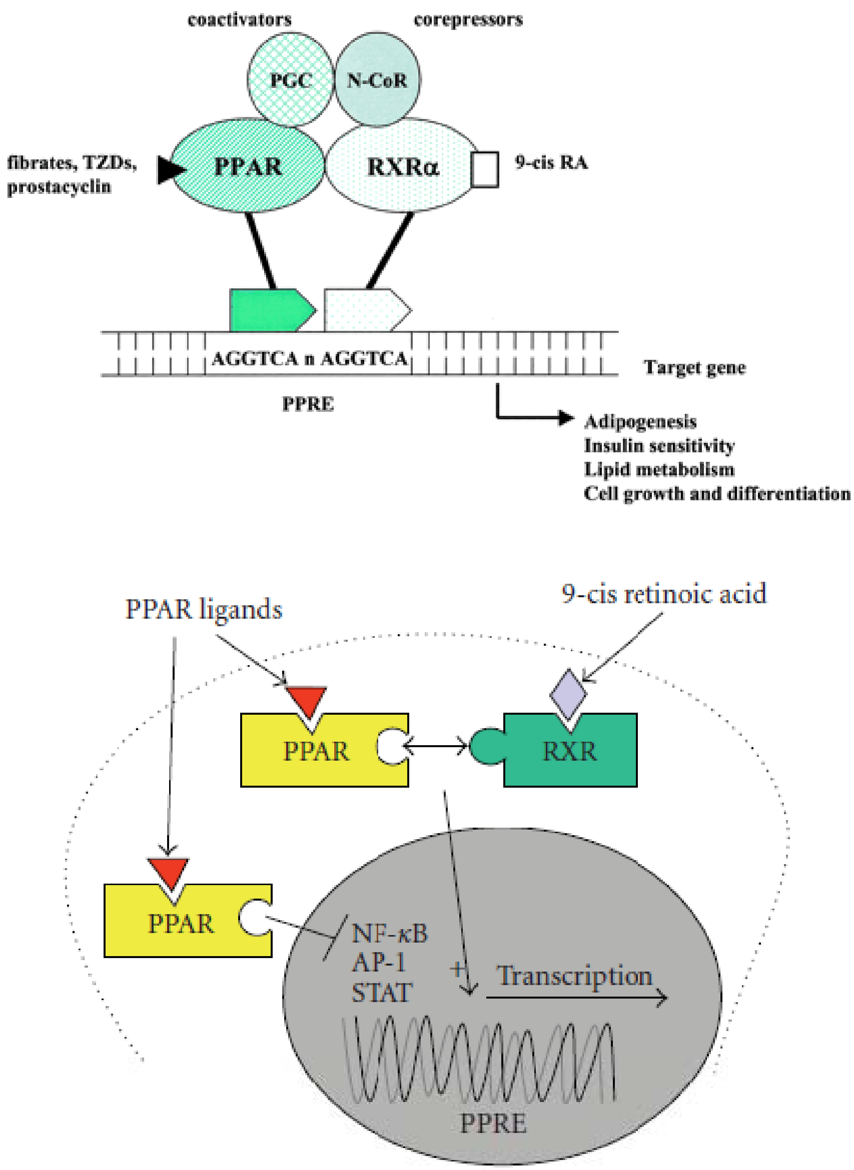


Figure 15. Transactivation and transrepression effects of peroxisome proliferator-activated receptors

2.7.4.1. Peroxisome proliferator-activated receptor α:

PPAR α is highly expressed in the vasculature, including the endothelial cells, smooth muscle cells, and macrophages. Activation of PPARα leads to modulation of lipid metabolism, including transcription apo A1 and AII, resulting in increased levels of "cardioprotective" HDL-C (Yusaku *et al*., 2007). β-oxidation and LPL activity are also stimulated following activation of PPAR α. This leads to a decrease in TG and free

fatty acids, and levels of apo C III; which inhibits LPL-mediated breakdown of TG, further resulting in lower TG levels (Vosper, *et al*., 2002). Finally, LDL-C particles are shifted from a small, dense to a large, buoyant form to create particles that are less atherogenic and more easily cleared (Paolo *et al*., 2003).

In addition PPAR α regulates apolipoprotein AV (Apo AV), phospholipid transfer protein (PLTP) and scavenger receptor class B, type I (SR-BI) in liver that are intimately involved in lipoprotein metabolism. PPARα dependent induction of the apo AV gene; an apolipoprotein component of HDL and LDL particles; could be the major factor contributing to the decrease of plasma TGs seen in response to fibrate treatment. In addition to their role in lipid homeostasis, PPARα agonists have anti-inflammatory actions and influence glucose metabolism (Beyer *et al*., 2008).

The natural ligands of PPARα include prostaglandins, leukotrienes, and medium- and long-chain free fatty acids such as eicosapentaenoic acid and docosahexenoic acid. Synthetic ligands of this receptor are utilized in the management of dyslipidaemia , and include members of the fibrate drug class (e.g., gemfibrozil, clofibrate, fenofibrate, and bezafibrate).

2.7.4.2. Peroxisome proliferator-activated receptors agonism and lipid lowering:

PPARα was recognised to be the target receptor for the fibrate class of anti-hyperlipidemic drugs, whereas PPARγ was shown to function as the cellular receptor of the thiazolidinedione (TZD) class of insulin-sensitising drugs. Notably, a recent study has shown that all the TZDs are not necessarily activators of PPARγ. In contrast to PPARα and PPARγ, there are no marketed drugs that target PPAR δ, and the physiological role of PPARδ remains largely mysterious due, in part, to the lack of selective ligands as chemical tools to study its pharmacology (Feige *et al*., 2006).

It was found that PPARα and PPARγ ligands protective against atherosclerosis and inhibit macrophages' foam cell formation. Studies have shown that PPAR agonists can serve as very good candidates for antihyperlipidemic. The therapeutic benefits of existing lipid-lowering drugs of the fibrate class are elicited through the activation of PPARα, resulting in the increased catabolism and decreased hepatic secretion of triglyceride-rich lipoproteins. Furthermore, a more recent study revealed that fibrates are not selective agonists of PPARα and their effects on other isoforms of PPARs are rather complex (Koyama *et al*., 2005).

3. Oxidation – hyperlipidemia:

A free radical is a molecule (or) molecular fragment that contains one (or) more unpaired electrons in its outer orbital and is conventionally represented by a superscript dot (R·). These are product of partial reduction of O_2 and are highly reactive in living system; hence, they are also called reactive O_2 species (or) ROS. Reactive oxygen species (ROS) such as superoxide anion ($O_2^{\cdot}$), hydrogen peroxide (H_2O_2), hydroxyl radicals (-$OH^{\cdot}$) and Lipid peroxide radicals ($ROO^{\cdot}$) are produced during normal cellular function and their high chemical reactivity leads to the oxidation of lipids, proteins or DNA. Production is counterbalanced by antioxidant enzymes such as superoxide dismutases (SOD), catalase, glutathione peroxidase, thioredoxins, and peroxiredoxins (Serdar *et al*., 2006). Therefore, the balance between ROS generation and antioxidant activity is critical to the pathogenesis of oxidative stress-related disorders (Papaharalambus and Griendling, 2007).

3.1. Pathology

Uncontrolled production of ROS often leads to damage of cellular macromolecules (DNA, protein, and lipids). It is well known that ROS can bring about damage to

proteins by site-specific damage at metal binding sites and disulfide bonds in addition to fragmentation, cross-linking, and aggregation, which may damage or change the functions of proteins (RuiLi *et al*., 2006). The unsaturated chain of membrane fatty acids can readily react with free radicals and undergo peroxidation. This process can become autocatalytic after initiation and yields lipid peroxides, lipid alcohols and aldehydic by-products such as 4-hydroxynonenal (4-HNE) and malondialdehyde (MDA). Other reactions of ROS with proteins result in the oxidation of various amino acid side chains, often leading to a loss of function (Manfredini *et al*., 2010). Increasing evidence, however, points to excess oxidant stress, causing a negative shift in redox balance and resulting in multiple abnormalities in gene responses. A significant part of the oxidant load *in-vivo* is due to advanced glycation end-products (AGEs) from proteins or advanced lipoxidation end-products. These products are ubiquitous and abundant within cells and in the extracellular milieu, constituting important contributors to ROS production and oxidant stress, redox sensitive transcription activity, and inappropriate inflammatory responses in disease. It has been shown that the diet can be an important exogenous source of excess oxidants (Liu *et al*., 2006).

Emerging evidence indicates that ROS are important risk factors in the pathogenesis of many diseases if the antioxidant system is impaired. The involvement of hydroxyl free radicals has been shown to be a major contributing factor for the peroxidative damage to lipoproteins present in the blood, which are responsible for the initiation and progression of atherosclerosis (Papaharalambus and Griendling, 2007). The oxidative modification hypothesis of atherosclerosis predicts that LDL oxidation is an early event in atherosclerosis and that oxidized LDL contributes to atherogenesis. Under oxidative stress, proteins as well as lipids are the major targets of ROS. Oxidative modification of proteins by ROS can cause loss of catalytic activity of the proteins and marks the

protein for subsequent proteolytic degradation. Damage to protein components of cells can lead to substantial decreases in the amounts of important enzymes and the accumulation of the damaged protein. Such damaged protein can seriously compromise cellular integrity (Figure 16) (Roberts and Sindhu, 2009).

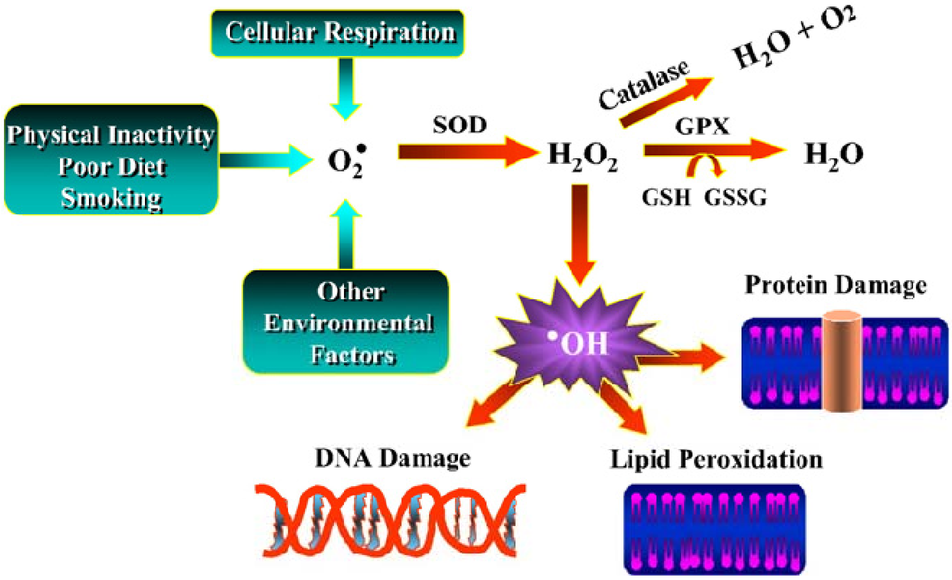


Figure 16. Overview of generation and antioxidant enzymatic detoxification of ROS.

Hence, hyperlipidemia and excess of free radicals formed during lipid peroxidation are the important risk factors in the initiation and progression of atherosclerosis. There is an increased awareness for the need to lower the elevated plasma lipoproteins, especially LDL and the oxidative stress due to excess of free radicals (Atul *et al*., 2010).

3.2. Antioxidants:

Antioxidants or free radicals scavengers are substances that inhibit oxidation (or) scavenge the activated oxygen species.

3.2.1. Mechanism of antioxidants:

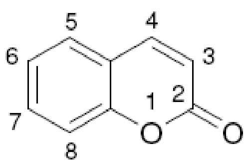
1. They inhibit the initial generation of free radicals
2. They decrease the intensity of free radical chain reactions

Antioxidants may inhibit atherogenesis and improve vascular function by different mechanisms. Enzymatic protection against ROS and the breakdown products of peroxidized lipids and oxidized protein and DNA are provided by many enzyme systems such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR) and glucose 6 phosphate dehydrogenase (G6PD) (Roberts and Sindhu, 2009).

Nonenzymatic detoxification against ROS is provided by lipid- and water-soluble antioxidants. Lipid-soluble antioxidants present in LDL include vitamin E and carotenoids, and water-soluble antioxidants present in the extracelluler fluid of the arterial wall include vitamin C. Furthermore, antioxidants present in the cells of the vascular wall decrease cellular production and release of ROS, inhibit endothelial activation and improve the biologic activity of endothelium-derived nitric oxide through a cell- or tissue-specific antioxidant action (Serdar *et al.*, 2006).

3.2.2. Examples of antioxidants:

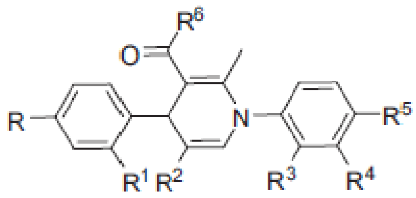
Many coumarin derivatives have special ability to scavenge ROS free radicals. They exhibited significant dual lipid lowering and antioxidant activity (Sashidhara *et al.*, 2008; Sashidhara *et al.*, 2010, a; Sashidhara *et al.*, 2010, b)



coumarin

In the following ongoing research towards the exploration of biologically important heterocycles, it was reported that organocatalyzed N-aryl-1,4-dihydropyridines,

compounds 2a, 2g, and 2i have shown promising antidyslipidemic activity whereas compound 2m and 2n have exhibited significant antioxidant activity (Atul *et al.*, 2010).



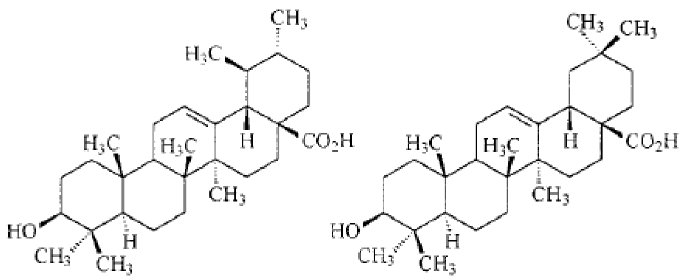
2

Entry	R	R^1	R^2	R^3	R^4	R^5	R^6	Product
1	H	H	H	H	H	H	OC_2H_5	**2a**
2	H	H	H	H	H	CH_3	OC_2H_5	**2b**
3	H	H	H	H	H	F	OC_2H_5	**2c**
4	H	H	H	H	H	Cl	OC_2H_5	**2d**
5	H	H	H	H	H	Br	OC_2H_5	**2e**
6	H	NO_2	H	H	H	H	OC_2H_5	**2f**
7	H	H	H	H	H	H	OCH_3	**2g**
8	H	H	CH_3	H	H	OCH_3	OCH_3	**2h**
9	H	H	CH_3	H	H	H	OCH_3	**2i**
10	OCH_3	H	H	H	H	H	OCH_3	**2j**
11	H	H	CH_3	H	Cl	H	OCH_3	**2k**
12	H	H	CH_3	CH_3	CH_3	H	OCH_3	**2l**
13	H	H	H	H	H	H	O^tBu	**2m**
14	H	H	H	H	H	Cl	O^tBu	**2n**

There have been reports that the lipid lowering drugs: fibrates, statins and bile acid sequestrants used for the treatment of hyperlipidemia associated disorders do not possess antioxidant property and they are also not free from toxic side effects, so research is increasingly focusing on natural alternative medicines that reduce blood lipid levels. A plant-based diet has potential for the treatment of hyperlipidemia, and generally has few or no side effects. Several studies have shown that polyphenol compounds (such as isoflavones or flavanoids found in soy, tea, or wines) are the most promising natural antioxidants with regard to the inhibition of atherogenic modifications of LDL (De-Santana *et al.*, 2008). For example results indicate that besides achieving antioxidant action *in-vitro*, the consumption of green tea increased the serum antioxidant defenses in normolipidemic and in dyslipidemic subjects (Camargo *et*

al., 2006). Anthocyanins and water extracts from mulberry fruit can scavenge free radicals, inhibit LDL oxidation, and have beneficial effects on blood lipid and atherosclerosis (Xiaolan *et al*., 2010).

Oleanolic acid (3b-hydroxy-olea-12-en-28-oic acid) and its isomer, ursolic acid (3b-hydroxy-urs-12-en-28-oic-acid) (Figure 17), are triterpenoid compounds that exist largely in food products (vegetable oils), and in more than 120 plant species. They are distributed in the natural plants in the form of free acid or aglycones for triterpenoid saponins, showed antioxidant and antihyperlipidemic activity (Somova *et al*., 2003).



URSOLIC ACID OLEANOLIC ACID

Figure 17. Chemical structures of ursolic acid and oleanolic acid.

In-vitro as well as *in-vivo* studies has been proven that *Benincasa cerifera* and *Punica granatum* flowers have an antioxidant and antihyperlipidemic activity (Bagri *et al*., 2009; Yagnik *et al*., 2009).

4. Rationale of the present work:

Atherosclerosis, the underlying cause of CHD that is the leading cause of death in most parts of the world, is a multifactorial disease. Several mechanisms participate in its initial manifestation and progression, mainly involving the triad: dyslipidaemia, oxidative stress, and inflammation. Dyslipidaemia, which can range from hyper-cholesterolemia to hyper-lipoproteinemia, is one of the many modifiable risk factors for CHD. Currently available anti-dyslipidaemic agents include statins, fibrates, nicotinic acids and bile acid sequestrants. Since these drugs are not only costy, but also have potential side effects, a search for alternative therapeutic agents was necessitated.

Furthermore, specific lipoprotein receptors are being discovered as targets for pharmacological intervention to correct lipoprotein disorders. Treatment issues remain regarding the appropriate degree of LDL cholesterol reduction and whether, in people with very high levels, it would be preferable to achieve the LDL cholesterol goal with a powerful statin alone or combined with an agent that lowers LDL cholesterol by a different mechanism.

The main focus in the development of novel agents is the patient with low HDL-C, usually associated with hypertriglyceridaemia. Another area of development involves agents that will lower both cholesterol and triglyceride levels. Future emphasis will be on correcting all lipid abnormalities for the prevention of CHD, not just lowering LDL cholesterol.

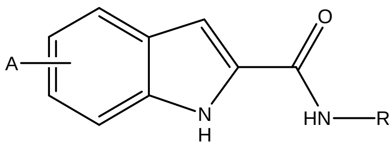
It is envisaged that, beside cholesterol lowering property, a hypolipidemic agent that incorporates antioxidant activity will be able to protect endothelial and myocardial function could serve as a better anti-atherosclerotic agent.

The present work is based on a previous search carried out by our group, reveals a class of novel heterocyclic carboxamide compounds acting as lipid lowering agents, therefore it is a promising candidate for the treatment of hyperlipidemia. This work is a continuation to the research team work which started with 5- methoxy-indole 2-carboxamide of aminobezophenone and aminoanthraquinone (Al-Qirim, *et al*., 2009) and their bioisoster benzofuran derivatives (Shattat, *et al*., 2010).

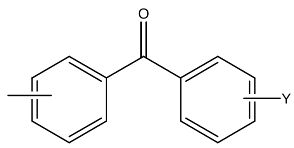
Towards an ongoing drug discovery programme for developing new antihyperlipidemic drugs, wherein we have synthesized compounds that exhibited lipid lowering and a potential antioxidant properties and thus represent a new class of dyslipidemic agents.

4.1. Aims and objectives:

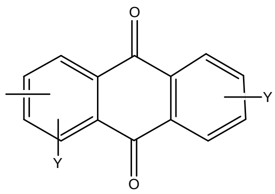
The aim of this work is to synthesize new heterocyclic, indole carboxamide derivatives of formula I, II, III and IV, starting from the "privileged" nucleus indole, as shown below in (Figure 18).



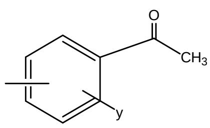
R= Formula I



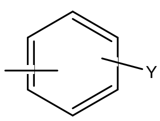
Formula II



Formula III



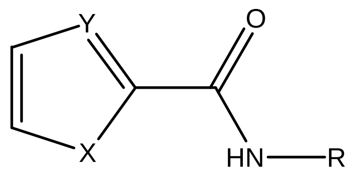
Formula IV



A = H, Cl, F, OH or OMe.
Y = H, R (alkyl), OR, NO_2, NH_2, COOH, Halogen.
Y = Fused benzene, OH, alkyl (IV)

Figure 18. General formulas for the synthesise of new heterocyclic, indole carboxamide derivatives

2. An extended aim is to prepare model compounds of another scaffold (Figure 19) to get the carboxamides analoguos derivatives of 5 membered ring π-excessive 2-carboxamides.



R = Formula I, II, III, IV above
X = N, O, N-Me
Y = H, C, fused benzene (Y=N)

Figure 19. A scaffold for the synthesise of carboxamides analoguos derivatives of 5 membered ring π-excessive 2-carboxamides

3. Characterization of all intermediates and final products by spectroscopic techniques (NMR, EA, IR and MS if possible).

4. *In-vivo* animal evaluation of therapeutic potentials of these compounds as anti-dyslipidimic and their comparison with the standard drugs in order to establish the activity.

5. *In-vitro* evaluation of therapeutic potentials of selected compounds for antioxidant activities.

6. Optimization and structural modifications to the active hits in order to increase the activity, or find new active derivatives.

7. Attempts to correlate these structures with the activity and establish an idea about SARs.

5. Literature Review:

The present work is based on the unexpected discovery of a class of novel indole carboxamide compounds, that act as inhibitors of cholesterol and triglycerides (Al-Qirim, *et al*., 2009; Shattat, *et al*., 2010). Therefore it is a promising candidate for the treatment of hyperlipidemia.

5.1. Introduction to indoles:

Indoles derivatives have been a topic of substantial research interest and continue to be one of the most active areas of heterocyclic chesmistry, particularly due to their natural occurance and broad pharmacological activities. A large number of indole derivatives are at the core as pharmacologically active lead compounds for drug development. The indole nucleus, known to chemists as benzopyrrole, is the parent member of a broad spectrum of nitrogen heterocyclic biochemicals commonly found in nature. It provides a useful privileged structure for the construction of many ligands structure (Lakshmi *et al*., 2010).

The indole scaffold probably represents one of the most important structural subunits for the discovery of new drug candidates. The demonstration that many alkaloids contain the indole nucleus, the recognition of the importance of essential amino acid tryptophan in human nutrition and the discovery of plant hormones served to bring about a massive search on indole chemistry, giving rise to a vast number of biologically active natural and synthetic products, with a wide range of therapeutic targets, such as anti-inflammatories, phosphodiesterase inhibitors, 5-hydroxytryptamine receptor agonists and antagonists, cannabinoid receptors agonists and HMG-CoA reductase inhibitors (Kent *et al*., 2004). Many of these target-receptors belong to the class of GPCRs (integral membrane G-protein coupled receptors) and possess a conserved binding pocket that is recognized by the indole scaffold in a "common" complementary

binding domain, explaining the great number of drugs that contain the indole substructure, such as indomethacin, ergotamine, frovatriptan, ondansetron, tadalafil, among many others (Kent *et al*., 2004).

The term "privileged structure" was first introduced in 1988. A privileged structure was defined as "a single molecular framework able to provide ligands for diverse receptors". Privileged structures with their inherent affinity for diverse biological targets, represent an ideal source of core scaffolds for the design of libraries targeted at various receptors. In addition, the likely drug-like properties of privileged structures and substructures might produce more drug-like compound libraries and leads. It was envisaged, that the privileged structures could be a valuable alternative in the search for new receptor ligands by suitably decorating these substructures. Since then, an increasing number of substructural frameworks have been described as privileged structures, including indoles, aryl piperazines, spiro phenylpiperidines, biphenyls, benzopyranes, 1,4-dihydropyridines, and pyrrole. These privileged structures have since then, deliberate or not, been used extensively in medicinal chemistry programs to identify new ligands, especially for G-protein coupled receptors (GPCR's) (Welsch *et al*., 2010).

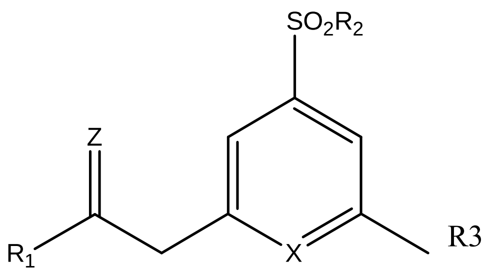
The indole framework is a medicinally relevant scaffold and has become widely identified as a privileged structure or pharmacophore. For instance, the non-steroidal anti-inflammatory drugs (NSAIDs) constitute the most important class of therapeutic agents for the treatment of inflammatory related diseases. Indomethacin, acemetacin and etodolac are NSAIDs possessing an indole ring, and their ROS and reactive nitrogen species (RNS) scavenging activity has already been reported. One of the most promising endowments displayed by indole derivatives is their antioxidant potential, conferred by their ability to scavenge ROS and RNS (Estevão *et al*., 2010; Lakshmi et al., 2010).

Indole structures are considered as privileged structure motifs, due to their ability to bind many receptors within the body. As a result, a great deal of research has been dedicated to incorporating the indole functionality in the design and synthesis of anti-hyperlipidemic and antioxidant compounds for the treatment of hyperlipidemia.

5.2. Indole carboxamide and related heterocyclic derivatives as a target for drug discovery:

The established pharmacological importance and the wide diversity of biological activities exhibited by heterocyclic carboxamide compounds prompted this search to synthesis novel indole-2-carboxamides derivatives of (Figure 18 and 19).

An invention relates to a preparation of 5-chloro-indole carboxamide and thieno [2,3-b] pyrrole carboxamide derivatives of formula I (Figure 20), reveals the use of these derivatives in the treatment of diabetes, insulin resistance, diabetic neuropathy, diabetic retinopathy, hypertension and atherosclerosis, etc [Bussolotti *et al.*, 2004, a].



(formula I)

R1 is (un)substituted indol-2-yl or 2-chlorothieno [2,3-b]pyrrol-5-yl.
R2 is alkyl substituted with 1-3 fluorine atoms.
R3 is H, NO2, NH2, or NH-alkyl, etc.
X is N, CH, or C-O-alkyl.
Z is O or S

Figure 20. General formula for the preparation of 5-chloro-indole carboxamide and thieno [2,3-b] pyrrole carboxamide derivatives

The invention of 5-chloro-indole-2-carboxamide (Figure 21), reveals that it inhibits glycogen phosphorylase, which is useful in the treatment of diabetes, insulin resistance, diabetic neuropathy, diabetic nephropathy, diabetic retinopathy, cataracts and hyperglycemia. It was found that it exhibits lowering effect for hypercholesterolemia, hypertension, hyperlipidemia, atherosclerosis, and tissue ischemia, particularly myocardial ischemia (Gammill and Ronald, 2003).

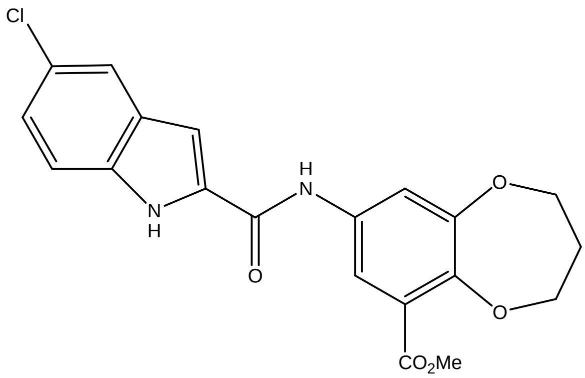
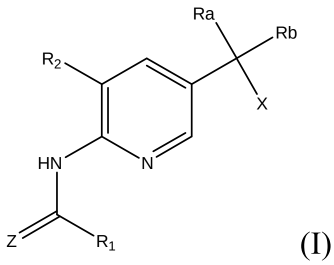


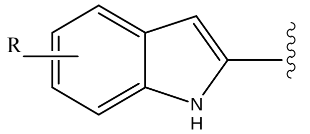
Figure 21. 5-chloro-indole-2-carboxamide formula for the inhibition of glycogen phosphorylase

The following invention provides title compounds I (Figure 22), the stereoisomers and prodrugs thereof, and the pharmaceutically acceptable salts of the compounds. This research indicates the use of compound I for the treatment of atherosclerosis, diabetes, insulin resistance, diabetic neuropathy, diabetic nephropathy, diabetic retinopathy, cataracts, hypercholesterolemia, hypertriglyceridemia, hyperlipidemia, hyperglycemia, hypertension, tissue ischemia, or myocardial ischemia. The compounds are found to be inhibitors of glycogen phosphorylase (Bussolotti *et al.*, 2004, b; Bussolotti *et al.*, 2004, c).

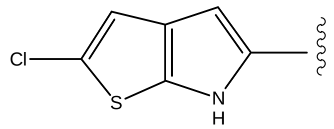


(I)

Q1 =



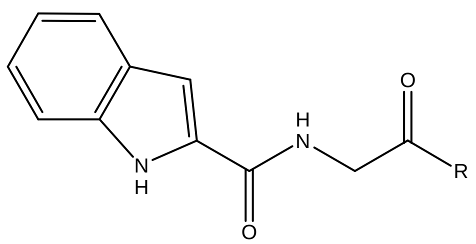
Q2 =



R1 = Q1 or Q2;
R = 1-3 of H, NH2, cyano, NO2, halo, alkyl, or alkoxy;
R2 = alkoxy;
Ra, Rb = Me or OH, provided that both are not OH simultaneously;
X = CH2OH, CO2Rc; Rc = H, alkyl, or CON (heterocycloalkyl);
Z = O or S

Figure 22. General formula for compounds that found to be inhibitors of glycogen phosphorylase

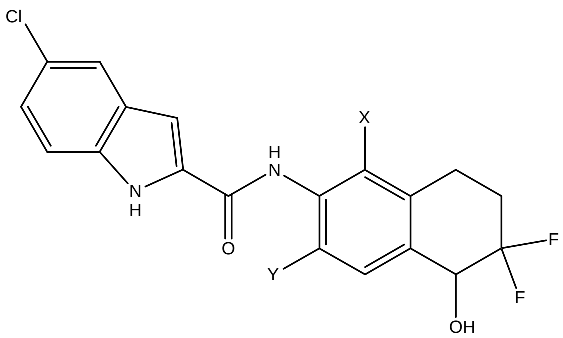
A recent research shown that indole-2-carboxylic amides act as inhibitors of human liver glycogen phosphorylase a (HLGPa) represented by the general formula I (Figure 23). The derivatives were found to be useful in the prophylactic or therapeutic treatment of diabetes (Guixia *et al.*, 2004), hyperglycemia, hyperlipidemia, hypertension, atherosclerosis or tissue ischemia and as cardio-protectants (Bradley *et al.*, 2006).



I

Figure 23. Formula I (indole-2-carboxylic amides) that act as inhibitor of human liver glycogen phosphorylase a

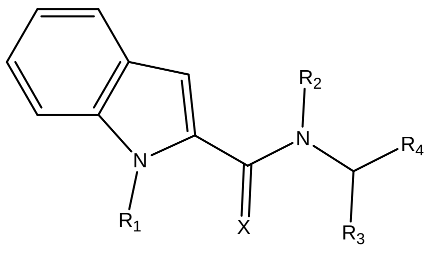
More recently, a novel series of N-bicyclo-5-chloro-1H-indole-2-carboxamide derivatives containing a hydroxy moiety was synthesized, and was proved that the optically active 5-hydroxy-5,6,7,8-tetrahydronaphthalene derivative (**25a-e**) (Figure 24), to be the most potent HLGPa inhibitor in this series (Onda *et al*., 2008).



a: X= H, Y= OMe;
b: X= OMe, Y= H;
c: X= H, Y= F;
d: X= F, Y= H;
e: X= Y= F.

Figure 24. A novel series of N-bicyclo-5-chloro-1H-indole-2-carboxamide derivatives that act as potent human liver glycogen phosphorylase a (HLGPa) inhibitor

Indole-carboxamides have other potential therapeutic activities. A new class of potent and selective monoamine oxidases (MAO) were synthesized as indole analogues and reported to be potent MAO-A or MAO-B inhibitors (Figure 25) (La Regina *et al*., 2008).



R_1-R_3= H, CH_3;
R_4= Ph, cyclohexyl;
N= 0-4;
X= O, H_2

Figure 25. General formula for an indole analogues that act as potent MAO-A or MAO-B inhibitors

The synthesis of the N-sulfonyl-2-indole carboxamide compounds (Figure 26) as PPAR-γ binding agents. Several of these compounds were found to stimulate osteoblast differentiation in a cell-based assay, thus suggesting a potential application for the treatment of osteoporosis (Hopkins *et al*., 2006).

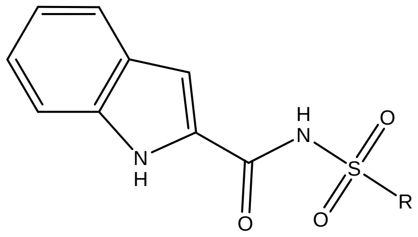


Figure 26. General formula for N-sulfonyl-2-indole carboxamide as PPAR-γ binding agents

The design of novel 3-indole sulfonamides as potent non-nucleoside reverse transcriptase inhibitors (NNRTIs) with balanced profiles against common Human immunodeficiency virus reverse transcriptase (HIV RT) (Figure 27), was also reported recently (Zhao *et al*., 2008).

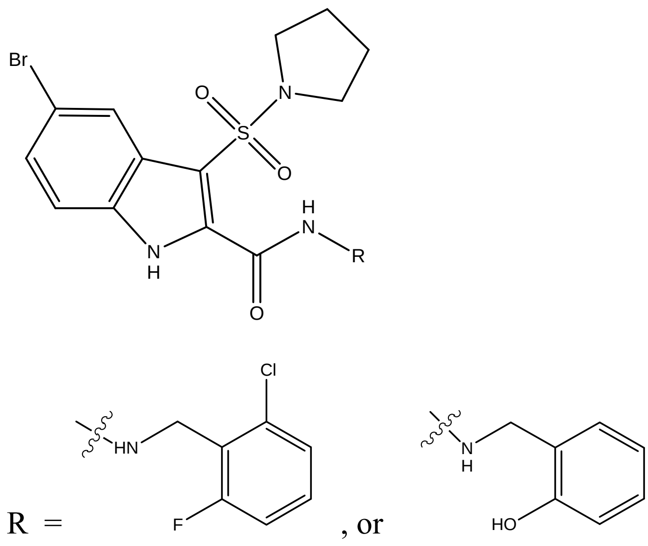
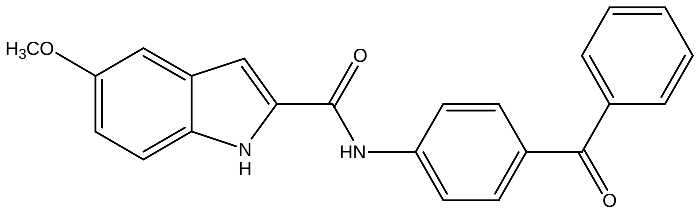
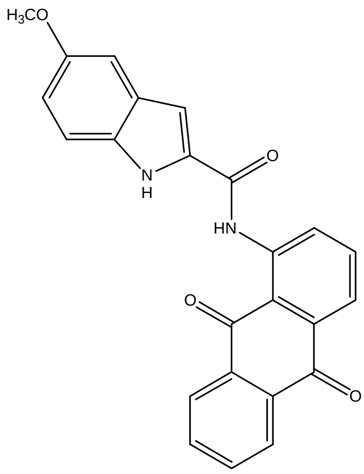


Figure 27. A novel formula for 3-indole sulfonamides as potent non-nucleoside reverse transcriptase inhibitors (NNRTIs)

This research work is a continuation of our research team work who started with 5-methoxy-indole 2-carboxamide of aminoanthraquinone and aminobezophenone of (Figure 28) below (Al-Qirim *et al.*, 2009).



(I)



(II)

Figure 28. The structures of formula I and II (5-methoxy-indole 2-carboxamide of aminoanthraquinone and aminobezophenone).

It was found that indole-2-carboxamide derivatives (I) and (II) improved lipid abnormalities such as hypertriglyceridemia and hypercholesterolemia, and then elevated the HDL levels in Triton-induced rats, suggesting that (I) and (II) may be useful in the treatment of patients with lipid abnormalities (Al-Qirim *et al.*, 2009).

Based on this research, this work involves investigation of new indole-2-carboxamide derivatives, as well as, other indole isosteric heterocycles such as pyrrole and furan.

6. Experimental part:

6.1. Synthesis of target compounds:

6.1.1. Materials:

All chemicals, reagents and solvents were purchased from commercial sources (Sigma-Aldrich, Riedel-de Haen, Fluka, Ubichem, Aldrich, Uniwise, Tedia and Merck Corporation) and were used in the experimentation without further purification. 1-H-indole-2-carboxylic acid, 1-methyl-indole-2-carboxylic acid, 1-H-furoyl-2-carboxylic acid, 1-H-pyrrole-2-carboxylic acid, 1-methyl pyrrole-2-carboxylic acid, 2-aminobenzophenone, 3-aminobenzophenone, 4-aminobenzophenone, 2-amino-4'-methylbenzophenon, 2-amino-5-chloro-benzophenone, 2-Aminobenzophenone-2'-carboxylic acid, 4-aminoacetophenon, 4-amino- 3,5-dichloro-acetophenon, 3-aminoacetophenon, 2-aminoacetophenon, 2-aminopyridine, 3-aminopyridine, 3,4-diaminopyridine, 2-aminobenzimidazole, p-chloroaniline, p-fluoroaniline, 4-methyl-benzylamine, 3-aminophenol and 2-methoxy-benzylamine were bought from (Across Organics, Belgium). 1- aminoanthraquinone, 2- aminoanthraquinone, 3-aminobenzoic acid, 3-aminophenyl acetic acid, 3-aminophenyl propionic acid, 4-(aminomethyl)-benzoic acid, phenylmagnesium chloride and silica gel were bought from (Aldrich, USA). 1-amino-5-chloro-anthraquinone and 1-amino-4-bromo-2-methyl anthraquinone were bought from (Uniwise International, China). 1-amino-4-hydroxy-anthraquinone was bought from (Ubichem, UK). P-toluidine and p-anisidine were bought from (Fluka, Switzerland). P-nitroaniline, hydrochloric acid and potassium carbonate were bought from (Riedel-De Haen, Germany). Benzene, chloroform, ethanol, acetone, dimethylsulphoxide, ethyl acetate, cyclohexane, pyridine, methanol and dichloromethane were bought from (Tedia, USA). Dimethylformamide and 3,4-

dichloroaniline were bought from (Merck, Germany). Magnesium sulfate anhydrous was bought from (Scharlau, Spain). Filter papers was bought from (Whatman, England).

6.1.2. Spectroscopic and other characterization techniques carried out for the synthesized compounds:

Nuclear magnetic resonance spectra (NMR) were recorded on a Varian Oxford-300 (300 MHz) spectrometer at faculty of pharmacy, University of Jordan, and on Bruker Ultra shield-300 (300 MHz), Al-Albeit University. Chemical shifts are reported in ppm related to tetramethylsilane (TMS) as the internal standard. Deuteriated dimethylsulfoxide (DMSO-d_6) and deuteriated chloroform ($CDCl_3$) were used as solvents in sample preparation, otherwise is indicated. ^{1}H NMR data are reported in the following way; chemical shift (ppm), (multiplicity, coupling constant (Hz), number of protons, the corresponding proton(s).

Infra red (IR) spectra were recorded using Shimadzu 8400F FT-IR spectrophotometer (Japan) at faculty of pharmacy, Al-Zaytoonah Private University. The samples were dissolved in chloroform ($CHCl_3$) and analyzed as thin solid films using NaCl plates or using potassium bromide (KBr) discs (Merk, Dermstadt, Germany).

Melting points (m.p) were determined in open capillaries on a Stuart scientific electro-thermal melting point apparatus at faculty of pharmacy, University of Jordan, and are uncorrected.

High resolution mass spectra (HRMS) were measured in positive or negative ion mode using electrospray ionization (ESI) technique by collision-induced dissociation on a Bruker APEX-4 (7 Tesla) instrument at department of chemistry, University of Jordan. The samples were dissolved in acetonitrile, diluted in spray solution (methanol/water 1:1 v/v + 0.1 formic acid) and infused using a syringe pump with a flow rate of

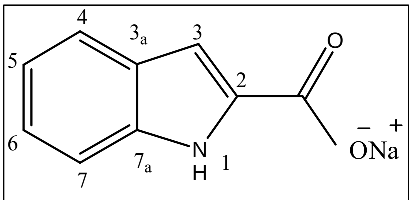
2µL/min. External calibration was conducted using Arginine cluster in a mass range m/z 175-871.

Elemental analysis (EA) of C, H and N were performed using Euro Elemental analyzer (Italy) at faculty of pharmacy, University of Jordan.

Thin layer chromatography (TLC) was performed on aluminum plates pre-coated with fluorescent silica gel GF254 from ALBET, (Germany). Silica Gel (70-230 Mesh) from GCC, (United Kingdom), was used for preparative TLC and plates were visualized under UV lamp, Spectroline cabinet, Model CX-20, (USA).

6.2. General procedure for the hydrolysis of esters:

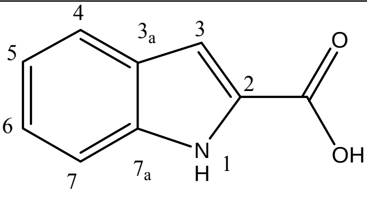
6.2.1. Sodium 1*H*- indole-2-carboxylate (2) (Guixia *et al.*, 2004):



2

A mixture of water (25 mL) and sodium hydroxide (NaOH, 3.49 g, 89.5 mmol) were added to a solution of the ethyl-indole-2-carboxylate (**1**, 6.78 g, 35.8 mmol) in methanol (25 mL), in a round-bottomed flask. The mixture was refluxed at room temperature (RT) for 2-3 hrs and let to cool. After evaporation of methanol a white solid indole carboxylate sodium salt was obtained (**2**, 6.46 g, 83%). ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 11.27 (br s, 1H, NH-indole), 7.46 (d, *J* = 7.8 Hz, 1H, H-7), 7.40 (d, *J* = 8.4 Hz, 1H, H-4), 7.01 (dd, *J* = 6.9, 7.8 Hz, 1H, H-6), 6.88 (dd, *J* = 7.2, 8.1 Hz, 1H, H-5), 6.70 (s, 1H, H-3).

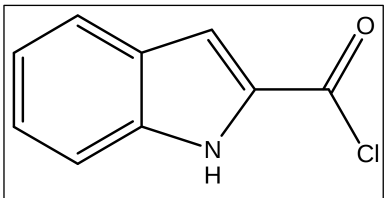
6.2.2. 1*H*-indole-2-carboxylic acid (3) (La Regina *et al.*, 2008):



3

The sodium indole salt (**2**) was treated with 2 N HCl in a mixture of 50% aqueous methanol 50 ml. The mixture was stirred for 2 hrs, upon cooling, the acid precipitated and the mixture was left until maximal precipitation has been obtained. The solid was filtered, washed three times with water (15 mL), and dried under reduced pressure to give indole-2- carboxylic acid (**3**) as a white crystals (5.70 g, 92%); m.p = 205-210; ^{1}H-NMR (300 MHz, DMSO-d$_6$): δ = 12.89 (br s, 1H, COOH), 11.71 (s, 1H, NH-indole), 7.62 (d, *J* = 8.1 Hz, 1H, H-7), 7.42 (d, *J* = 8.1 Hz, 1H, H-4), 7.21 (dd, *J* = 7.2, 7.5 Hz, 1H, H-6), 7.07 (s, 1H, H-3), 7.04 (dd, *J* = 7.8, 7.5 Hz, 1H, H-5); ^{13}C-NMR (DMSO-d$_6$): δ = 163.46 (CO-acid), 137.90, 129.07, 127.52, 124.91, 122.59, 120.61, 113.14, 107.95 ppm; IR (thin film): ν = 3352 (NH-indole), 3055 (COOH), 1709 (CO), 1620, 1520, 1439, 1420, 1346, 1238, 1196, 1119, 772, 745 cm^{-1}. This compound was bought from Across for further use.

6.3. Synthesis of indole-2-carbonyl chloride (4) (Hopkins *et al.*, 2006):



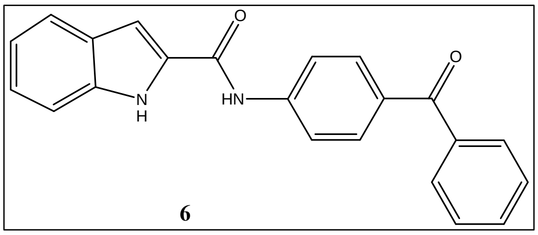
4

A mixture of indole-2-carboxylic acid (**3**, 1 g, 6.2 mmol) and thionyl chloride ($SOCl_2$) (3.6 mL, 50 mmol) in 25 mL of dry chloroform ($CHCl_3$) and drops of N,N-dimethylformamide (DMF) was added in a round bottomed flask . The reaction mixture was refluxed for 18 hrs at 70–80 °C till the completion of the reaction. Solvent was evaporated under vacuum, then $CHCl_3$ was added twice and evaporated again to dryness in order to remove the excess of $SOCl_2$ present, to afford acid chloride (**4**, 0.95 g , 84%) as a greenish yellow precipitate; R_f:

0.86 (cyclohexane/ ethylacetate, 7:3); m.p.= 115-120°C; ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 11.90 (s, 1H, NH-indole), 7.65 (d, *J* = 7.8 Hz, 1H, H-7), 7.47 (d, *J* = 8.1 Hz, 1H, H-4), 7.26 (dd, *J* = 7.2, 7.8 Hz, 1H, H-6), 7.16 (s, 1H, H-3), 7.07 (dd, *J* = 7.5, 7.2 Hz, 1H, H-5) ppm.

6.4. Synthesis of unsubstituted indole-benzophenone 2-carboxamides group (Series A):

Synthesis of N-[4-benzoylphenyl]-1*H*-indole-2-carboxamide (6):

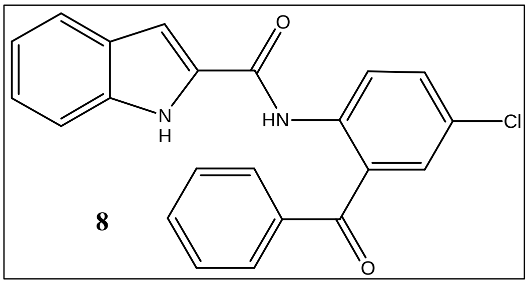


A. Ester pathway: Ethyl-1*H*-indole-2-carboxylate (**1,** 0.5 g, 2.6 mmol) was treated with 4-aminobenzophenone (**5,** 1.5 g, 7.6 mmol) in the presence of sodium ethoxide ($NaOC_2H_5$, 0.16 g, 2.4 mmol) and DMF (5 mL). The mixture of reaction was refluxed for 24 hrs at 150 °C and then filtered. DMF was removed by evaporation under reduced pressure and the residue was purified by column chromatography using $CHCl_3$/MeOH (99:1) as eluent to afford the title compound as a yellow solid (**6**, 0.11 g, 11%); R_f = 0.83 ($CHCl_3$/MeOH, 97:3).

B. Acyl pathway: Indole-2-carbonyl chloride (**4**, 0.39 g, 2.2 mmol) was added to a solution of 4-aminobenzophenone (**5**, 0.42 g, 2.1 mmol) and $NaOC_2H_5$ (0.35 g, 5.14 mmol) in 20 ml dry dichloromethane (DCM). The mixture was refluxed for 12 hrs at 80 °C, then cooled, washed with 1N HCl, followed by 1M sodium hydroxide and brine. The organic layer was taken, dried over magnesium sulfate ($MgSO_4$) anhydrous and filtered. The organic layer was removed by evaporation under reduced pressure and the residue was purified by column chromatography using chloroform / methanol (95:5) as eluent to afford a dark off-white solid (**6**, 0.230 g, 32%); R_f: 0.77 ($CHCl_3$/MeOH, 9:1); m.p.: 220-222 °C; ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 11.85 (br s, 1H, NH-indole),

10.59 (s, 1H, NHCO), 8.05 (d, *J*= 8.75 Hz, 1H, Ar-H), 7.82 (d, *J*= 8.74 Hz, 1H, Ar-H), 7.79-7.64 (m, 5H, Ar-H), 7.63 – 7.45 (m, 5H, Ar-H), 7.25 (dd, *J* = 8.03, 7.12 Hz, 1H, Ar-H), 7.09 (dd, *J* = 7.06, 7.12 Hz, 1H, Ar-H); ^{13}C-NMR (DMSO-d_6): δ = 195.08 (CO-ketone), 160.53 (CONH), 160.53, 143.77 (CH-Ar), 138.04, 137.51 (CH-Ar), 132.73 (CH-Ar), 132.37 (CH-Ar), 131.99, 131.81, 131.56 (CH-Ar), 131.49, 129.88 (CH-Ar), 129.62, 128.97 (CH-Ar), 127.42 (CH-Ar), 124.59 (CH-Ar), 122.38 (CH-Ar), 120.52 (CH-Ar), 119.65 (CH-Ar), 119.23, 112.93 (CH-Ar), 105.16 (CH-Ar) ppm; IR (thin film): ν = 3341 (NH-indole), 3298 (NHCO), 1659 (br CO), 1639, 1589, 1528, 1404, 1319, 1281, 1238, 1199, 1177, 926, 829, 741 cm^{-1}; HRMS (ESI, negative mode): m/z (M^+ - H^+) 339.11387 ($C_{22}H_{15}N_2O_2$) requires 339.11335; Anal. Calcd for $C_{22}H_{16}N_2O_2$: C, 77.63; H, 4.74; N, 8.23. Found: C, 77.41; H, 4.66; N, 8.24.

Synthesis of N-[2-(benzoyl-4-chlorophenyl)]-1*H*-indole-2-carboxamide (8):

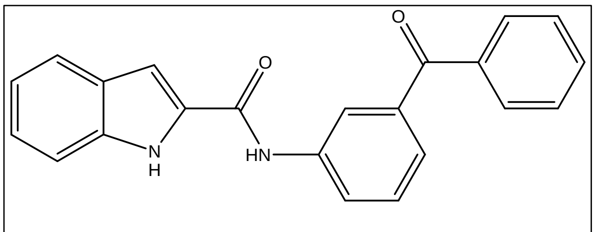


A. Ester pathway: Ethyl-2-indole-carboxylate (**1**, 1.0 g, 5.3 mmol) was treated with 2-amino-5-chloro-benzophenone (**7**, 3.7 g, 15.9 mmol) in the presence of $NaOC_2H_5$ (0.36 g, 5.3 mmol) and DMF (5 mL). The mixture of reaction was refluxed for 24 hrs at 150 °C and then filtered. DMF was removed by evaporation under reduced pressure and the residue was purified by column chromatography using cyclohexane/EtOAc (9:1) as eluent to afford the title compound as a brown oil (**8**, 0.32 g, 15 %); R_f = 0.93 ($CHCl_3$).

B. Acyl pathway: Indole-2-carbonyl chloride (**4**, 0.40 g, 2.2 mmol) was added to a solution of 2-amino-5-chlorobenzophenone (**7**, 0.41 g, 1.8 mmol) and ($NaOC_2H_5$, 0.4 g, 5.88 mmol) in 20 ml dry DCM. The mixture was refluxed for 18 hrs at 80 °C, then cooled, washed with 1N HCl, followed by 1M sodium hydroxide and brine. The organic layer was taken, dried over $MgSO_4$ anhydrous and filtered. The organic layer was

removed by evaporation under reduced pressure and the residue was purified by column chromatography using chloroform / methanol (95:5) as eluent to afford a brownish yellow solid (**8**, 0.250 g, 38%); R_f: 0.85 ($CHCl_3$/MeOH, 9:1); m.p.= 263-265 °C; ^1H-NMR (300 MHz, DMSO-d_6): δ = 11.70 (br s, 1H, NH-indole), 10.75 (s, 1H, NHCO), 7.79 - 7.70 (m, 4H, Ar-H), 7.67 – 7.55 (m, 2H, Ar-H), 7.54 – 7.34 (m, 4H, Ar-H), 7.23 – 7.14 (m, 2H, Ar-H), 7.08 – 7.00 (m, 1H, Ar-H); ^{13}C-NMR (DMSO-d_6): δ = 194.17 (CO-ketone), 159.96 (CONH), 137.33, 136.99, 135.59, 133.32 (CH-Ar), 133.04, 132.09 (CH-Ar), 130.98, 130.24 (CH-Ar), 129.84 (CH-Ar), 128.75 (CH-Ar), 128.69, 127.29, 126.34 (CH-Ar), 124.44 (CH-Ar), 122.26 (CH-Ar), 120.44 (CH-Ar), 112.82 (CH-Ar), 104.49 (CH-Ar) ppm; IR (thin film): ν = 3306 (NH-indole), 3248 (NHCO), 2936, 1632 (br CO), 1601, 1578, 1528, 1420, 1400, 1312, 1288, 1246, 1200, 1157, 949, 826, 760 cm^{-1}; HRMS (ESI, negative mode): m/z (M^+ - H^+) 373.07728 ($C_{22}H_{14}ClN_2O_2$) requires 373.07438; Anal. Calcd for $C_{22}H_{15}ClN_2O_2$: C, 70.50; H, 4.03; N,7.47. Found: C, 70.12; H, 3.92; N, 7.57.

Synthesis of N-[3-benzoylphenyl]-1*H*-indole-2-carboxamide (10):

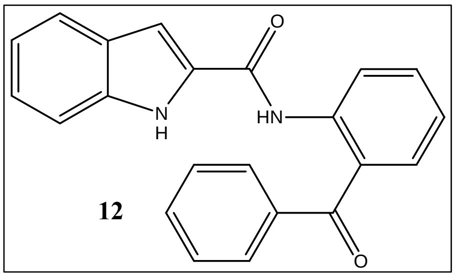


10

A. Ester pathway: Ethyl-1*H*-indole-2-carboxylate (**1,** 0.5 g, 2.6 mmol) was treated with 3-amino-benzophenone (**9,** 1.54 g, 7.8 mmol) in the presence of $NaOC_2H_5$ (0.16 g, 2.4 mmol) and dry DMF (5 mL). The mixture of reaction was refluxed for 24 hrs at 150 °C and then filtered. DMF was removed by evaporation under reduced pressure and the residue was purified by column chromatography using $CHCl_3$/MeOH (99:1) as eluent to afford the title compound as a pale brown oil (**10**, 0.16 g, 16%); R_f = 0.55 ($CHCl_3$/MeOH, 96:4).

B. Acyl pathway: Indole-2-carbonyl chloride (**4**, 0.32 g, 1.8 mmol) was added to a solution of 3-aminobenzophenone (**9**, 0.35 g, 1.8 mmol) and $NaOC_2H_5$ (0.36 g, 5.30 mmol) in 20 ml dry DCM. The mixture was refluxed for 8 hrs at 80 °C, then cooled, washed with 1N HCl, followed by 1M sodium hydroxide and brine. The organic layer was taken, dried over $MgSO_4$ anhydrous and filtered. The organic layer was removed by evaporation under reduced pressure and the residue was purified by column chromatography using chloroform / methanol (95:5) as eluent to afford a pale yellow solid (**10**, 0.135 g, 23%); R_f: 0.81 ($CHCl_3$/MeOH, 9:1); m.p.: 226-229 °C; ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 11.83 (br s, 1H, NH-indole), 10.52 (s, 1H, NHCO), 8.28 (s, 1H, Ar-H), 8.19 (d, *J*= 7.04 Hz, 1H, Ar-H), 7.80 (d, *J*= 6.88 Hz, 1H, Ar-H), 7.74-7.30 (m, 9H, Ar-H), 7.24 (dd, *J*= 6.38, 7.74 Hz, 1H, Ar-H), 7.08 (dd, *J*= 7.11, 6.64 Hz, 1H, Ar-H); ^{13}C-NMR (DMSO-d_6): δ = 196.13 (CO-ketone), 160.43 (CONH), 139.69, 137.91, 137.56, 137.38, 133.16 (CH-Ar), 131.63, 130.11 (CH-Ar), 129.54 (CH-Ar), 129.06 (CH-Ar), 127.46, 125.08 (CH-Ar), 124.39 (CH-Ar), 122.29 (CH-Ar), 121.55 (CH-Ar), 120.44 (CH-Ar), 112.87 (CH-Ar), 104.79 (CH-Ar) ppm; IR (thin film): ν = 3364 (NH-indole), 3291 (NHCO), 3051, 1647 (br CO), 1585, 1543, 1435, 1315, 1285, 1242, 1204, 860, 750 cm^{-1}; HRMS (ESI, negative mode): m/z (M^+ - H^+) 339.11390 ($C_{22}H_{15}N_2O_2$) requires 339.11335; Anal. Calcd for $C_{22}H_{16}N_2O_2$: C, 77.63; H, 4.74; N, 8.23. Found: C, 77.41; H, 4.49; N, 8.09.

Synthesis of N-[2-benzoylphenyl]-1*H*-indole-2-carboxamide (12):



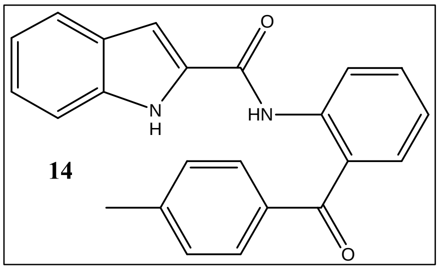
12

A. Ester pathway: Ethyl-1*H*-indole-2-carboxylate (**1,** 0.5 g, 2.6 mmol) was treated with 2-aminobenzophenone (**11,** 1.54 g, 7.8 mmol) in the presence of $NaOC_2H_5$ (0.16 g, 2.4 mmol) and dry DMF (5 mL). The mixture of reaction was refluxed for 4 days at 100 °C and then filtered. DMF was removed by evaporation under reduced pressure and the residue was purified by column chromatography using $CHCl_3$/EtOH (99:1) as eluent to afford the title compound as a yellow solid (**12**, 0.16 g, 16%); R_f = 0.84 ($CHCl_3$/MeOH, 98:2).

B. Acyl pathway: Indole-2-carbonyl chloride (**4**, 0.36 g, 2.0 mmol) was added to a solution of 2-aminobenzophenone (**11**, 0.39 g, 2.0 mmol) and $NaOC_2H_5$ (0.36 g, 5.30 mmol) in 20 ml dry DCM. The mixture was refluxed for 12 hrs at 80 °C, then cooled, washed with 1N HCl, followed by 1M sodium hydroxide and brine. The organic layer was taken, dried over $MgSO_4$ anhydrous and filtered. The organic layer was removed by evaporation under reduced pressure and the residue was purified by column chromatography using chloroform / methanol (95:5) as eluent to afford a spongy yellow solid (**12**, 0.145 g, 21%); R_f: 0.84 ($CHCl_3$/MeOH, 98:2); m.p.: 226-229 °C; ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 11.75 (br s, 1H, NH-indole), 10.89 (s, 1H, NHCO), 7.89 (d, *J*= 8.0 Hz, 1H, Ar-H), 7.77 (d, *J*= 8.3 Hz, 1H, Ar-H), 7.72-7.56 (m, 4H, Ar-H), 7.55-7.43 (m, 3H, Ar-H), 7.40 (d, *J*= 8.24 Hz, 1H, Ar-H), 7.31 (dd, *J*= 7.95, 6.30 Hz, 1H, Ar-H), 7.25-7.15 (m, 2H, Ar-H), 7.03 (dd, *J*= 7.11, 1.0 Hz, 1H, Ar-H); ^{13}C-NMR (DMSO-d_6): δ = 196.33 (CO-ketone), 159.96 (CONH), 137.78, 137.34 (CH-Ar), 137.29 (CH-Ar), 133.0 (CH-Ar), 132.75 (CH-Ar), 131.38 (CH-Ar), 131.14 (CH-Ar), 130.32, 130.24 (CH-Ar), 128.67 (CH-Ar), 127.37 (CH-Ar), 124.44 (CH-Ar), 124.38 (CH-Ar), 123.97 (CH-Ar), 122.25 (CH-Ar), 120.44 (CH-Ar), 112.84 (CH-Ar), 104.15 (CH-Ar) ppm; IR

(thin film): ν = 3302 (NH-indole), 3188 (NHCO), 1667 (br CO), 1632, 1582, 1535, 1447, 1312, 1258, 1157, 930, 810, 745 cm^{-1}; HRMS (ESI, negative mode): m/z (M^+ - H^+) 339.11390 ($C_{22}H_{15}N_2O_2$) requires 339.11335;Anal. Calcd for $C_{22}H_{16}N_2O_2$: C, 77.63; H, 4.74; N, 8.23. Found: C, 77.91; H, 4.49; N, 8.41.

Synthesis N-[2-(4-toloylphenyl)]-1*H*-indole-2-carboxamide (14):



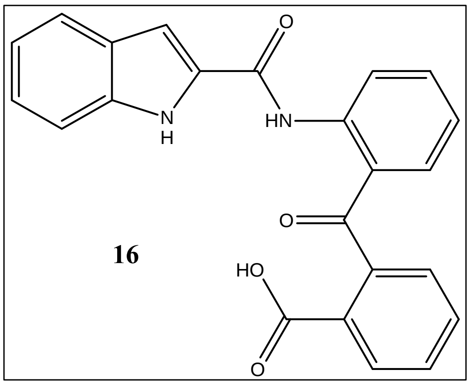
14

A. Ester pathway: Ethyl-1*H*-indole-2-carboxylate (**1,** 0.3 g, 1.6 mmol) was treated with 2-Amino-4'-methylbenzophenone (**13,** 1.0 g, 4.7 mmol) in the presence of $NaOC_2H_5$ (0.10 g, 1.5 mmol) and dry DMF (3 mL). The mixture of reaction was refluxed for 3 days at 150 °C and then filtered. DMF was removed by evaporation under reduced pressure and the residue was purified by column chromatography using cyclohexane/EtOAc (85:15) as eluent to afford the title compound as a yellow solid (**14**, 0.12 g, 20%); R_f = 0.75 ($CHCl_3$/MeOH, 98:2).

B. Acyl pathway: Indole-2-carbonyl chloride (**4**, 0.40 g, 2.2 mmol) was added to a solution of 2-Amino-4'-methylbenzophenone (**12**, 0.40 g, 1.9 mmol) and $NaOC_2H_5$ (0.26 g, 3.82 mmol) in 20 ml dry DCM. The mixture was refluxed for 8 hrs at 80 °C, then washed with 1N HCl, followed by 1M sodium hydroxide and brine. The organic layer was taken, dried over $MgSO_4$ anhydrous and filtered. The organic layer was removed by evaporation under reduced pressure and the residue was purified by column chromatography using chloroform / methanol (95:5) as eluent to afford an orange-yellow solid (**14**, 0.380 g, 56%); R_f: 0.75 ($CHCl_3$/MeOH, 98:2); m.p.: 252-254 °C; ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 11.72 (br s, 1H, NH-indole), 10.87 (s, 1H, NHCO), 7.90 (d, *J*= 7.9 Hz, 1H, Ar-H), 7.70-7.61 (dd, *J*=8.1, 2.4 Hz, 4H, Ar-H), 7.48-7.37 (m,

2H, Ar-H), 7.34-7.26 (dd, *J*= 7.8, 2.5 Hz, 3H, Ar-H), 7.21 (dd, *J*= 8.7, 5.5 Hz, 2H, Ar-H), 7.06 (dd, *J* = 7.1, 6.9 Hz, 1H, Ar-H), 2.35 (s, 3H, CH_3); ^{13}C-NMR (DMSO-d_6): δ = 196.05 (CO-ketone), 159.94 (CONH), 143.42, 137.33, 137.27, 135.13, 132.58 (CH-Ar), 131.42, 131.08 (CH-Ar), 130.47 (CH-Ar), 129.25 (CH-Ar), 127.39, 124.38 (CH-Ar), 123.95 (CH-Ar), 122.24 (CH-Ar), 120.45 (CH-Ar), 112.83 (CH-Ar), 104.12 (CH-Ar), 21.60 (CH-Ar) ppm; IR (thin film): ν = 3449 (NH-indole), 3302 (NHCO), 2900, 1659 (br CO), 1624, 1601, 1582, 1535, 1447, 1423, 1315, 1296, 1265, 1242, 1199, 1161, 1126, 930, 833, 806, 760, 741 cm^{-1}; HRMS (ESI, negative mode): m/z (M^+ - H^+) 353.12955 ($C_{23}H_{17}N_2O_2$) requires 353.12900; Anal. Calcd for $C_{23}H_{18}N_2O_2$: C, 77.95; H, 5.12; N, 7.90. Found: C, 78.02; H, 5.21; N, 8.02.

Synthesis of N-[2-(2-hydroxycarbonol benzoyl phenyl)]-1*H*-indole-2-carboxamide (16):



16

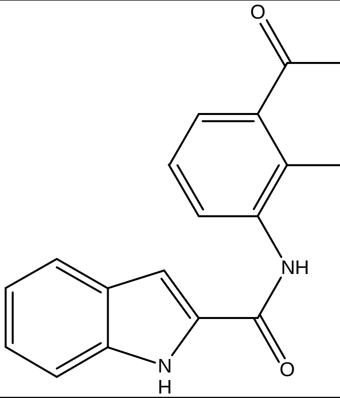
A. Ester pathway: Ethyl-1*H*-indole-2-carboxylate (**1,** 0.26 g, 1.4 mmol) was treated with 2-aminobenzophenone-2'-carboxylic acid (**15,** 1.5 g, 6.2 mmol) in the presence of $NaOC_2H_5$ (0.07 g, 1.0 mmol) and dry DMF (4 mL). The mixture of reaction was refluxed for 24 hrs at 150 °C and then filtered. DMF was removed by evaporation under reduced pressure and the residue was purified by column chromatography using $CHCl_3$ as eluent to afford the title compound as an off-white solid (**16**, 0.2 g, 35%); R_f = 0.75 (100% $CHCl_3$).

B. Acyl pathway: Indole-2-carbonyl chloride (**4**, 0.36 g, 2.00 mmol) was added to a solution of 2-aminobenzophenone-2'-carboxylic acid (**15**, 0.38 g, 1.6 mmol) and $NaOC_2H_5$ (0.70 g, 10.29 mmol) in 20 ml dry $CHCl_3$. The mixture was refluxed for 24 hrs at 80 °C, then cooled, washed with 1N HCl, followed by 1M sodium hydroxide and

brine. The organic layer was taken, dried over $MgSO_4$ anhydrous and filtered. The organic layer was removed by evaporation under reduced pressure and the residue was purified by column chromatography using chloroform / methanol (95:5) as eluent to afford a yellow solid (**16**, 0.305 g, 50%); R_f: 0.75 (100% $CHCl_3$); m.p.: 339-340 °C; ^1H-NMR (300 MHz, DMSO-d_6): δ = 14.21 (br s, 1H, COOH), 12.42 (br s, 1H, NH-indole), 11.82 (s, 1H, NHCO), 8.53 (d, *J*= 8.2 Hz, 1H, Ar-H), 7.86-7.80 (m, 1H, Ar-H), 7.69 (d, *J*= 7.94 Hz, 1H, Ar-H), 7.57-7.45 (m, 2H, Ar-H), 7.44-7.36 (m, 2H, Ar-H), 7.28-7.15 (m, 4H, Ar-H), 7.12-7.00 (m, 2H, Ar-H); ^{13}C-NMR (DMSO-d_6): δ = 202.12 (CO-ketone), 169.18 (COOH), 159.90 (CONH), 142.14, 140.10, 139.25, 137.51, 132.67 (CH-Ar), 132.24, 131.46 (CH-Ar), 129.69 (CH-Ar), 128.85 (CH-Ar), 128.81 (CH-Ar), 128.31, 127.58, 125.32 (CH-Ar), 124.36 (CH-Ar), 122.72 (CH-Ar), 122.26 (CH-Ar), 121.04 (CH-Ar), 120.50 (CH-Ar), 112.93 (CH-Ar), 103.84 (CH-Ar) ppm; IR (thin film): ν = 2600-3500 (OH), 3435 (NH-indole), 3318 (NHCO), 3071, 1670 (br CO), 1624 (CO), 1605, 1585, 1535, 1450, 1423, 1385, 1312, 1269, 1200, 1157, 933, 814, 748 cm^{-1}; HRMS (ESI, negative mode): m/z (M^+ - H^+) 383.10373 ($C_{23}H_{15}N_2O_4$) requires 383.10318; Anal. Calcd for $C_{23}H_{16}N_2O_4$: C, 71.87; H, 4.20; N, 7.29. Found: C, 71.71; H, 3.99; N, 6.95.

6.5. Synthesis of unsubstituted indole-anthraquinone carboxamides group (Series B):

Synthesis of N-(9,10-dihydro-9,10-dioxoanthracen-1-yl)-1*H* indole- 2-carboxamide (18):



18

Procedure A: Ethyl-indole-2-carboxylate (**1**, 0.80 g, 4.2 mmol) was treated with 1-aminoanthraquinone (**17**, 0.40 g, 1.8 mmol) in the presence of $NaOC_2H_5$ (0.33g, 4.83 mmol) and DMF (15 ml). The mixture was refluxed for 3 days at 150 °C and then filtered. DMF was removed by evaporation under reduced pressure and the residue was purified by column chromatography using cyclohexane/ethylacetate (85:15) then recrystalization from cyclohexane afforded a pale green solid (**18**, 30 mg, 5%).

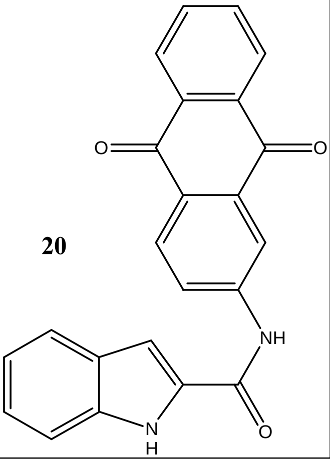
Procedure B: Indole-2-carbonyl chloride (**4**, 0.50 g, 2.8 mmol) was added to a solution of 1-aminoanthraquinone (**17**, 0.50 g, 2.2 mmol) and triethylamine (TEA, 1.3 mL, 9.3 mmol) in dry DMF 10 ml. The reaction mixture was stirred for 24 hrs at RT. DMF and the excess TEA were evaporated under reduced pressure, and the residue was stirred for 10 minutes in $CHCl_3$ and applied on a short chromatography column ($CHCl_3$:MeOH, 93:7). The fractions containing the product were collected and the solvent was removed under reduced pressure and the product dried in vacuo to afford a pale green solid (**18**, 55 mg, 7%).

Procedure C: Indole-2-carboxylic acid (**3**, 0.50 g, 3.1 mmol) was stirred with ice bath solution of 1,3-dicyclohexylcarbodiimide (DCC, 1.0 mmol) in anhydrous tetrahydrofuran (THF) 10 ml for 1 hr. The selected 1-aminoanthraquinone (**17**, 0.58 g, 2.59 mmol) was then added and mixed with the reaction solution. After stirring at RT

for 24 hrs, the mixture was filtered and concentrated under vacuum. The residue was applied over a column chromatography using cyclohexane/ethylacetate (85:15) and recrystallized (ethyl acetate/hexane) to afford a green solid (**18**, 40 mg, 4%), which was confirmed by NMR spectra to be the side product (dicyclohexane urea) as the major product. Data for side product (**A**, dicyclohexane urea): ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 5.58 (d, *J*= 7.8 Hz, 2H, NH), 2.48 (br s, 2H, CH-NH), 1.81-1.55 (m, 8H), 1.54 - 1.42 (m, 2H), 1.35-0.99 (m, 10H); ^{13}C-NMR (DMSO-d_6): δ = 157.08 (C=O), 47.96 (CH), 33.81 (CH_2), 25.75 (CH_2), 24.92 (CH_2).

Procedure D: A mixture of 1-aminoanthraquinone (**17**, 0.40 g, 1.8 mmol) and indole-2-carbonyl chloride (**4**, 0.40 g, 2.2 mmol) was refluxed at 120 °C for 18 hrs in air condenser, then 1,4-dioxane (15 mL) was added. The reaction mixture was stirred for additional 24 hrs at RT (Gouda *et al*., 2010 procedure adopted). The separated product was filtered, washed with ethanol, dried and crystallized from chloroform to afford a brown-green solid (**18**, 0.30 g, 45%); R_f: 0.59 (cyclohexane / ethylacetate, 6:4); m.p.: □ 350°C (decomposed); ^{1}H-NMR (300 MHz, DMSO-d_6): rotamers δ = 13.23 (br s, 1H, NH-indole), 12.08 (s, 1H, NHCO), 9.15 + 9.13 (2s, 1H, Ar-H), 8.50-7.76 (m, 5 H, Ar-H), 7.62-6.89 (m, 5 H, Ar-H) ; ^{13}C-NMR (DMSO-d_6): δ = 182.60 (CO-ketone), 176.31 (CO-ketone), 163.21 (CONH), 134.37 (CH-Ar) , 133.90 (CH-Ar) , 132.90, 131.68, 129.37, 128.06 (CH-Ar), 127.69, 127.13, 126.48 (CH-Ar), 125.25, 124.47, 123.85 (CH-Ar), 122.37 (CH-Ar), 121.83, 120.90 (CH-Ar), 118.16 (CH-Ar), 116.20 (CH-Ar), 115.24 (CH-Ar), 112.94 (CH-Ar), 104.18 (CH-Ar) ppm; IR (thin film): ν = 3314 (NH-indole), 3213 (NHCO), 3125, 1667 (br CO), 1636 (CONH), 1578, 1531, 1412, 1312, 1269, 1238, 1173, 1015, 806, 741, 705 cm^{-1}; HRMS (ESI, negative mode): m/z (M^{+} - H^{+}) 365.09317 ($C_{23}H_{13}N_2O_3$) requires 365.09262; Anal. Calcd for $C_{23}H_{14}N_2O_3$: C, 75.40; H, 3.85; N, 7.65. Found: C, 75.19; H, 4.01; N, 7.96.

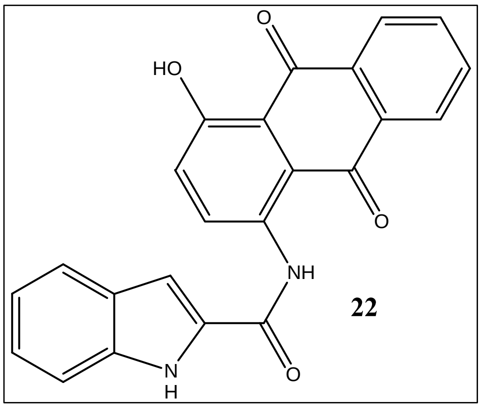
Synthesis of N-(9,10-dihydro-9,10-dioxoanthracen-2-yl)-1*H* indole- 2-carboxamide (20):



20

A mixture of 2-aminoanthraquinone (**19**, 0.45 g, 2.0 mmol) and indole-2-carbonyl chloride (**4**, 0.50 g, 2.8 mmol) was refluxed at 120 °C for 18 hrs in air condenser, then 1,4-dioxane (15 mL) was added. The reaction mixture was stirred for additional 24 hrs at RT. The separated product was filtered, washed with ethanol, dried and crystallized from chloroform to give a pale green solid (**20**, 0.46 g, 63%); R_f: 0.41 (cyclohexane / ethylacetate, 6:4); m.p.: □ 350°C (decomposed); ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 11.88 (br s, 1H, NH-indole), 10.79 (s, 1H, NHCO), 8.68 (d, *J* = 3.0 Hz, 1H, Ar-H), 8.39 (d, *J* = 9.0 Hz, 1H, Ar-H), 8.31-8.11 (m, 3H, Ar-H), 8.0-7.83 (m, 2H, Ar-H), 7.75 (d, *J* = 6.0 Hz, 1H, Ar-H), 7.56 (s, 1H, Ar-H), 7.46 (d, *J* = 6.0 Hz 1H, Ar-H), 7.37-7.03 (m, 2H, Ar-H); ^{13}C-NMR (DMSO-d_6): δ = 183.0 (CO-ketone), 181.86 (CO-ketone), 160.67 (CONH), 145.19, 137.65, 135.03 (CH-Ar), 134.68 (CH-Ar), 134.58, 133.67, 131.20, 128.89 (CH-Ar), 128.50, 127.43, 127.21 (CH-Ar), 127.13 (CH-Ar), 124.94 (CH-Ar), 124.77 (CH-Ar), 122.48 (CH-Ar), 120.58 (CH-Ar), 117.21 (CH-Ar), 112.96 (CH-Ar), 105.59 (CH-Ar) ppm; IR (thin film): ν = 3402 (NH-indole), 3321 (NHCO), 3117, 3067, 1667 (br CO), 1651 (CONH), 1589, 1535, 1416, 1331, 1292, 1238, 954, 840, 755, 710 cm^{-1}; HRMS (ESI, negative mode): m/z (M^+ - H^+) -365.09317 ($C_{23}H_{13}N_2O_3$) requires 365.09262; Anal. Calcd for $C_{23}H_{14}N_2O_3$: C, 75.40; H, 3.85; N, 7.65. Found: C, 75.72; H, 3.74; N, 7.87.

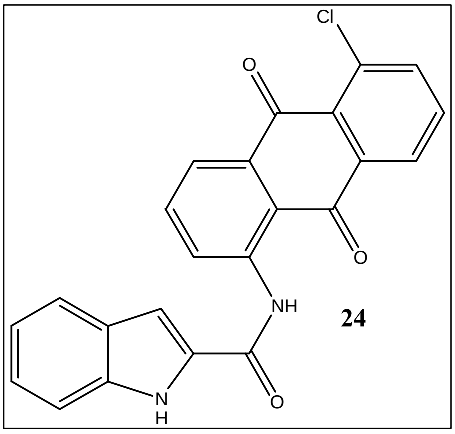
Synthesis of N-(1-hydroxy-9,10-dihydro-9,10-dioxo-anthracen-4-yl)-1*H* indole- 2-carboxamide (22):



22

A mixture of 1-amino-4-hydroxy-anthraquinone (**21**, 0.40 g, 1.7 mmol) and indole-2-carbonyl chloride (**4**, 0.40 g, 2.2 mmol) was refluxed at 120 °C for 18 hrs in air condenser, then 1,4-dioxane (15 mL) was added. The reaction mixture was stirred for an additional 24 hrs at RT. The separated product was filtered, washed with ethanol, dried and crystallized from chloroform to give a black solid (**22**, 0.32 g, 49%); R_f: 0.80 ($CHCl_3$/ MeOH/ Acetic acid, 94: 5: 1); m.p.: □ 350°C (decomposed); IR (thin film): ν = 2500-3450 (OH), 3310 (NH-indole), 1659 (br CO), 1624 (CONH), 1574, 1531, 1501, 1466, 1250, 1165, 1065, 741 cm^{-1}; HRMS (ESI, negative mode): m/z (M^+ - H^+) 381.08808 ($C_{23}H_{13}N_2O_4$) requires 381.08753; Anal. Calcd for $C_{23}H_{14}N_2O_4$: C, 72.52; H, 3.69; N, 7.33. Found: C, 72.20; H, 3.48; N, 7.52. The NMR results of this compound could not be obtained because of solubility problems.

Synthesis of N-(1-chloro-9,10-dihydro-9,10-dioxo-anthracen-5-yl)-1*H* indole- 2-carboxamide (24):

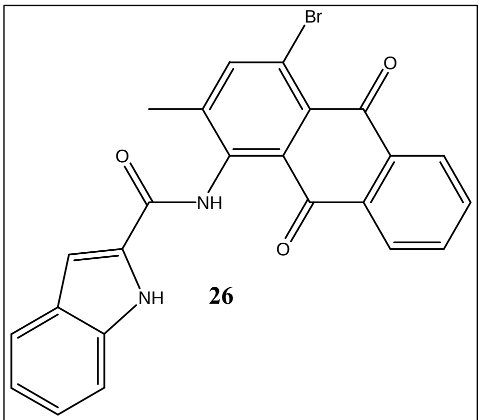


24

A mixture of 1-amino-5-chloro-anthraquinone (**22**, 0.45 g, 1.7 mmol) and indole-2-carbonyl chloride (**4**, 0.45 g, 2.5 mmol) was refluxed at 120 °C for 18 hrs in air condenser, then 1,4-dioxane (15 mL) was added. The reaction mixture was stirred for additional 24 hrs at RT. The separated product

was filtered, washed with ethanol, dried and crystallized from chloroform to give an orange solid (**24**, 0.45 g, 65%); R_f: 0.48 (cyclohexane / ethylacetate, 6:4); m.p.: □ 350°C (decomposed); ^{1}H-NMR (300 MHz, DMSO-d_6): rotamers δ = 13.03, 12.89 (2br s, 1H, NH-indole), 12.08, 11.72 (2s, 1H, NHCO), 9.12, 9.15 (2s, 1H, Ar-H), 8.38, 8.40 (2s, 1H, Ar-H), 8.21-7.78 (m, 5 H, Ar-H), 7.71-7.01 (m, 4 H, Ar-H); ^{13}C-NMR (DMSO-d_6): rotamers δ = 184.21 (CO-ketone), 179.10 (CO-ketone), 174.25 + 169.95 (CONH), 145.40, 138.13, 136.71, 135.10, 132.41, 127.49, 125.94, 125.10, 124.22, 123.77, 122.61, 122.0, 121.85, 121.0, 118.33, 113.21, 104.19 ppm; IR (thin film): ν = 3310 (NH-indole), 3213 (NHCO), 3071, 2986, 1674 (br CO), 1636 (CONH), 1578, 1528, 1431, 1412, 1312, 1261, 1022, 806, 745 cm^{-1}; HRMS (ESI, negative mode): m/z (M^+ - H^+) 399.05419 ($C_{23}H_{12}ClN_2O_3$) requires 399.05364; Anal. Calcd for $C_{23}H_{13}ClN_2O_3$: C, 68.92; H, 3.27; N, 6.99. Found: C, 69.02; H, 3.28; N, 7.03.

Synthesis of N-(1-bromo-3-methyl-9,10-dihydro-9,10-dioxo-anthracen-4-yl)-1*H* indole- 2-carboxamide (26):



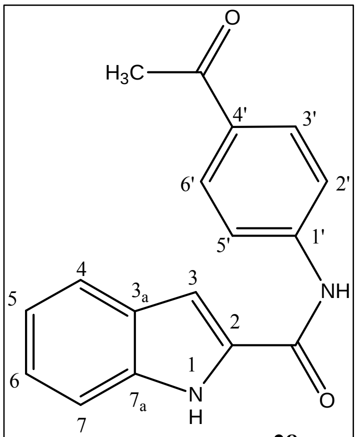
26

A mixture of 1-amino-4-bromo-2-methyl-anthraquinone (**25**, 0.40 g, 1.3 mmol) and indole-2-carbonyl chloride (**4**, 0.40 g, 2.2 mmol) was refluxed at 120 °C for 18 hrs in air condenser, then 1,4-dioxane (15 mL) was added. The reaction mixture was stirred for an additional 24 hrs at RT. The separated product was filtered, washed with ethanol, dried and crystallized from chloroform to give a black solid (**26**, 90 mg, 16%); R_f: 0.77 ($CHCl_3$/ MeOH/ Acetic acid, 94: 5: 1); m.p.: □ 350°C (decomposed); ^{1}H-NMR (300 MHz, DMSO-d_6): rotamers δ = 12.90 (br s, 1H, NH-indole), 11.85, 11.79 (2s, 1H, NHCO),

10.64 (s, 1H, Ar-H), 8.15 (s, 1H, Ar-H), 8.12-7.05 (m, 8H, Ar-H), 2.38 (s, 3H, CH_3); ^{13}C-NMR (DMSO-d_6): rotamers δ = 184.41 + 184.34 (CO-ketone), 181.85 + 181.79 (CO-ketone), 176.30, 163.30 + 160.16 (CONH), 142.49, 141.76, 139.04, 134.77, 126.76, 126.46, 125.91, 124.73, 124.42, 122.40, 121.81, 120.55, 120.41, 114.42, 112.90, 107.75, 104.94, 18.99 (CH_3) ppm; IR (thin film): ν = 3287 (NH-indole), 3179 (NHCO), 3063, 2920, 2855, 1678 (br CO), 1639 (CONH), 1524, 1489, 1458, 1420, 1308, 1258, 1238, 1119, 1076, 872, 818, 748, 718 cm^{-1}; HRMS (ESI, negative mode): m/z (M^+ - H^+) 457.01933 ($C_{24}H_{14}BrN_2O_3$) requires 457.01878; Anal. Calcd for $C_{24}H_{15}BrN_2O_3$: C, 62.76; H, 3.29; N, 6.10. Found: C, 62.93; H, 3.17; N, 6.35.

6.6. Synthesis of unsubstituted indole-acetophenone carboxamides and derivatives (Series C):

Synthesis of N-(4-acetylphenyl)-1*H*-indole-2-carboxamide (28):



28

Procedure A: Ethyl-indole-2-carboxylate (**1**, 0.85 g, 4.5 mmol) was treated with 4-aminoacetophenone (**27**, 0.41 g, 3.0 mmol) in the presence of sodium methoxide (0.05 g, 0.92 mmol) and dry DMF (10 ml). The mixture was refluxed for 2 days at 150 °C and then filtered. DMF was removed by evaporation under reduced pressure and the residue was purified by column chromatography using cyclohexane/ethylacetate (85:15) then recrystalization was carried out by chloroform / methanol to afford a red solid (**28**, 30 mg, 4%).

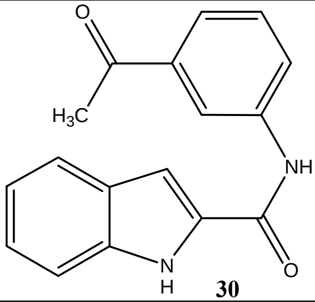
Procedure B: To a solution of 4-aminoacetophenone (**27**, 0.80 g, 5.9 mmol) in dry (THF, 5 ml) was added a 1.6 M hexane solution of Butyl lithium (BuLi, 4.2 ml, 6 mmol) at 0 °C and the mixture was stirred at RT for 1 hour. After cooling to -78 °C,

ethyl-indole-2-carboxylate (**1**, 0.96 g, 5.0 mmol) was added dropwise and the stirring was continued at this temperature for an additional 1 hour. The reaction was quenched with saturated NH_4Cl and workup was performed with ethyl acetate (Ooi *et al*., 1999 procedure was adopted). The organic extracts were collected then dried over anhydrous $MgSO_4$ and concentrated. Purification of the residue by column chromatography on silica gel (hexane/ ethyl acetate) gave a red solid (**28**, 60 mg, 4%).

Procedure C: Para-aminoacetophenone (**27**, 0.76 g, 5.6 mmol) was dissolved in a mixture of dry DCM (15 mL) and TEA (7.0 mmol). The solution was cooled with an ice-bath and the corresponding indole-2-carbonyl chloride (**4**, 0.50 g, 2.8 mmol) was added drop wise. The ice-bath was removed and the reaction was stirred at RT for 24 hrs, the solution was washed with 2 M HCl (40 mL), a 10% (w/v) solution of Na_2CO_3 (40 mL), and brine (25 mL) (Antwerpen *et al*., 2008 procedure was adopted). After drying and filtration, the solvent was evaporated under reduced pressure and the residue was purified by column chromatography using chloroform / methanol (95:5) as eluent to afford a red solid (**28**, 0.160 g, 21%); R_f: 0.48 (cyclohexane / ethylacetate, 6:4); m.p.: 229-231 °C (decomposed); ^{1}H-NMR (300 MHz, DMSO-d_6): rotamers δ = 11.82 + 11.79 (2br s, 1H, NH-indole), 10.52 + 10.50 (2br s, 1H, NHCO), 8.04-7.98 (m, 3H, Ar-H), 7.97 (s, 1H, Ar-H), 7.68 (dd, *J*= 9.0, 2.7 Hz, 1H, H-2', H-6'), 7.48 (d, *J*= 8.1 Hz, 2H, Ar-H), 7.22 (dd, *J*= 8.1, 2.7 Hz, 1H, Ar-H), 7.08 (dd, *J*= 8.0, 2.7 Hz 1H, Ar-H), 2.49 (s, 3H, $COCH_3$) ; ^{13}C-NMR (DMSO-d_6): rotamers δ = 196.99 + 197.02 (COCH3), 160.48 + 160.46 (CO amide), 143.92, 137.51, 132.37, 131.95, 131.53, 130.97, 129.82 (CH-Ar), 124.56 (CH-Ar), 122.34 (CH-Ar), 120.50 (CH-Ar), 119.67, 119.69 (CH-Ar), 112.91 (CH-Ar), 105.19 (CH-Ar), 26.89 + 26.29 (COCH3) ppm; IR (thin film): ν = 3383 (NH-indole), 3294 (NHCO), 1667 (br CO), 1593, 1535, 1404, 1358, 1319, 1269, 1242, 1180, 957, 833 cm^{-1}; HRMS (ESI, negative mode): m/z (M^+ - H^+) 277.09980

($C_{17}H_{13}N_2O_2$) requires 277.09770; Anal. Calcd for $C_{17}H_{14}N_2O_2$: C, 73.37; H, 5.07; N, 10.07. Found: C, 73.17; H, 4.97; N, 10.16.

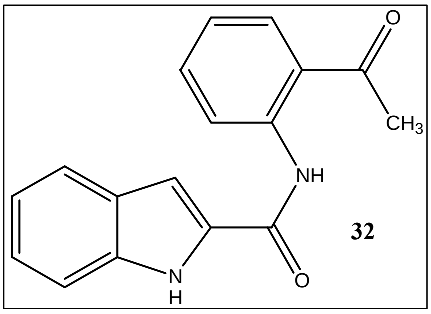
Synthesis of N-(3-acetylphenyl)-1*H*-indole-2-carboxamide (30):



Meta-aminoacetophenone (**29**, 0.45 g, 3.3 mmol) was dissolved in a mixture of dry DCM (15 mL) and TEA (7.0 mmol). The solution was cooled with an ice-bath and the corresponding indole-2-carbonyl chloride (**4**, 0.50 g, 2.8 mmol) was added dropwise. The ice-bath was removed and the reaction was carried out at RT for 18 hrs. Then, the solution was washed with 2 M HCl (40 mL), a 10% (w/v) solution of Na_2CO_3 (40 mL), and brine (25 mL). After drying and filtration, the solvent was evaporated under reduced pressure and the residue was purified by column chromatography using chloroform / methanol (95:5) as eluent to afford a brown solid (**30**, 0.200 g, 26%); R_f: 0.63 (cyclohexane/ethylacetae, 7:3); m.p.: 228-230 °C; ^{1}H-NMR (300 MHz, DMSO-d_6): rotamers δ = 11.75 (br s, 1H, NH-indole), 10.44 (br s, 1H, NHCO), 8.39 (s, 1H, Ar-H), 8.16 (d, *J*= 7.35 Hz, 1H, Ar-H), 7.71 (dd, *J*= 8.02, 8.36 Hz, 2H, Ar-H), 7.61-7.35 (m, 3H, Ar-H), 7.25 (dd, *J*= 7.34, 6.94 Hz, 1H, Ar-H), 7.08 (dd, *J*= 7.23, 6.84 Hz, 1H, Ar-H), 2.61 (s, 3H, $COCH_3$); ^{13}C-NMR (DMSO-d_6): δ = 198.17 (CO-ketone), 160.38 (CONH), 139.87, 137.76, 137.37, 131.65, 129.63 (CH-Ar), 127.47, 124.95 (CH-Ar), 124.40 (CH-Ar), 123.98 (CH-Ar), 122.29 (CH-Ar), 120.44 (CH-Ar), 119.78 (CH-Ar), 112.88 (CH-Ar), 104.64 (CH-Ar), 27.25 (CH-Ar) ppm; IR (thin film): ν = 3364 (NH-indole), 3294 (NHCO), 3055, 1663 (br CO), 1605, 1547, 1485, 1315, 1269, 1242, 1195, 940, 790, 752 cm^{-1}; HRMS (ESI, negative mode): m/z (M^+ - H^+) 277.09825 ($C_{17}H_{13}N_2O_2$) requires 277.09770; Anal.

Calcd for $C_{17}H_{14}N_2O_2$: C, 73.37; H, 5.07; N, 10.07. Found: C, 73.03; H, 4.98; N, 9.91.

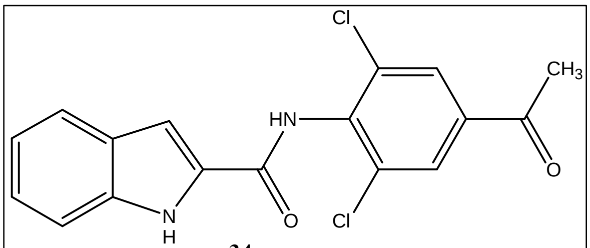
Synthesis of N-(2-acetylphenyl)-1*H*-indole-2-carboxamide (32):



32

Ortho-aminoacetophenone (**31**, 0.42 g, 3.1 mmol) was dissolved in a mixture of dry DCM (15 mL) and TEA (7.0 mmol). The solution was cooled with an ice-bath and the corresponding indole-2-carbonyl chloride (**4**, 0.45 g, 2.5 mmol) was added dropwise. The ice-bath was removed and the reaction was carried out at RT for 48 hrs. Then, the solution was washed with 2 M HCl (40 mL), a 10% (w/v) solution of Na_2CO_3 (40 mL), and brine (25 mL). After drying and filtration, the solvent was evaporated under reduced pressure and the residue was purified by column chromatography using chloroform / methanol (95:5) as eluent to afford an off-white solid (**32**, 0.165 g, 24%); R_f: 0.71 ($CHCl_3$/ MeOH/ Acetic acid, 94: 5: 1); m.p.: 215-218 °C; ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 10.91 (br s, 1H, NH-indole), 8.51 (s, 1H, NHCO), 7.48 (d, *J* = 7.8 Hz, 2H, Ar-H), 7.36 (d, *J* = 8.15 Hz, 2H, Ar-H) , 7.03 (dd, *J* = 7.03, 7.11 Hz, 2H, Ar-H), 6.91 (dd, *J* = 7.04, 6.95 Hz, 2H, Ar-H), 6.58 (s, 1H, Ar-H), 2.54 (s, 3H, $COCH_3$); ^{13}C-NMR (DMSO-d_6): δ = 198.21 (CO-ketone), 161.82 (CONH), 141.21, 139.92, 139.05, 136.06, 133.56, 132.95, 128.34, 124.91, 121.64 (CH-Ar), 121.05 (CH-Ar), 120.06 (CH-Ar), 118.88 (CH-Ar), 112.29 (CH-Ar), 102.03 (CH-Ar), 27.81 ($COCH_3$) ppm; IR (thin film): ν = 3449 (NH-indole), 3422 (NHCO), 1624 (CO), 1610 (CO), 1593, 1424, 1339, 1107, 789, 755 cm^{-1}; HRMS (ESI, negative mode): m/z (M^+ - H^+) 277.09817 ($C_{17}H_{13}N_2O_2$) requires 277.09770; Anal. Calcd for $C_{17}H_{14}N_2O_2$: C, 73.37; H, 5.07; N, 10.07. Found: C, 73.47; H, 4.88; N, 10.18.

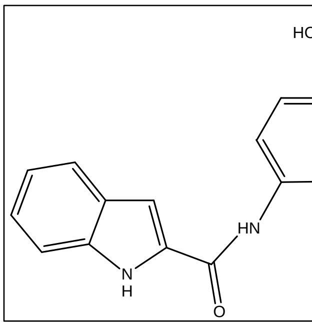
Synthesis of N-(4-acetyl-2,6-dichlorophenyl)-1*H*-indole-2-carboxamide (34):



34

Para-amino-3,5 dichloro-acetophenone (**33**, 0.42 g, 2.1 mmol) was dissolved in a mixture of dry DCM (15 mL) and TEA (7.0 mmol). The solution was cooled with an ice-bath and the corresponding indole-2-carbonyl chloride (**4**, 0.45 g, 2.5 mmol) was added dropwise. The ice-bath was removed and the reaction was carried out at RT for 3 days. Then, the solution was washed with 2 M HCl (40 mL), a 10% (w/v) solution of Na_2CO_3 (40 mL), and brine (25 mL). After drying and filtration, the solvent was evaporated under reduced pressure and the residue was purified by column chromatography using chloroform / methanol (95:5) as eluent to afford an off-white solid (**34**, 0.165 g, 23%); R_f: 0.86 ($CHCl_3$/ MeOH/ Acetic acid, 94: 5: 1); m.p.: 326-327 °C; ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 11.21 (br s, 1H, NH-indole), 8.59 (br s, 1H, NHCO), 8.09-7.80 (2m, 2H, Ar-H), 7.22-7.64 (m, 5H, Ar-H), 2.75 (br s, 3H, $COCH_3$); ^{13}C-NMR (DMSO-d_6): δ = 197.52 (CO-ketone), 151.21 (CONH), 129.56, 128.12, 125.21, 124.35, 118.52, 117.22, 25.62 ($COCH_3$) ppm; IR (thin film): ν = 3449 (NH-indole), 3101 (NHCO), 1697 (br CO), 1558, 1451, 1366, 1342, 1196, 1076, 879, 733 cm^{-1}; Anal. Calcd for $C_{17}H_{14}N_2O_2$: C, 73.37; H, 5.07; N, 10.07. Found: C, 73.47; H, 4.98; N, 10.18.

Synthesis of N-[4-(1-hydroxy-1-phenylethyl)phenyl]-1*H*-indole-2-carboxamide (36):

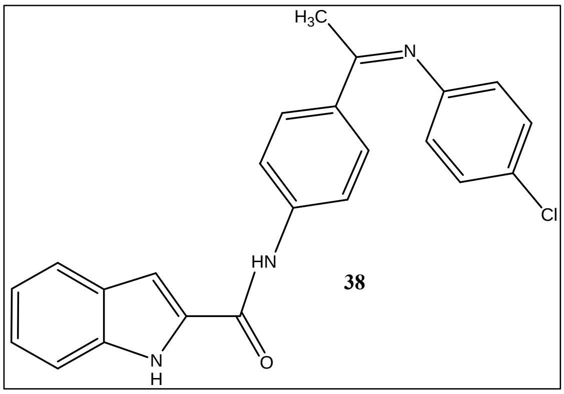


36

Procedure A: 4-aminoacetophenone (**27**, 0.50 g, 3.7 mmol) in 25 ml DCM, was treated with 10 drops of conc. HCl and stirred for 30 minutes in round bottom flask. The precipitate was filtered off, collected and dried (**27s**, 0.45 g, 2.6 mmol). The salt was suspended in 20 ml dry ether, placed in a round bottom flask and stirred at RT. Phenylmagnesium chloride (**GR,** 1.66 ml, 1.69 g, 12.4 mmol) dissolved in 20 ml dry ether, was added drop wise to the mixture with stirring on an ice bath over 20 minutes. Then, the reaction mixture was refluxed for additional 4 hrs at RT. Next, saturated aq. ammonium chloride salt (10 ml) was added and the mixture was extracted with ether (30 ml, 3x). The organic layer was washed with brine and dried with $MgSO_4$, concentrated by evaporating the solvent. The crude mixture have shown that the alcohol derivative (**27_{salt}**, 120 mg, 0.48 mmol) was produced in low amount. Next, the product (**27_{salt}**) was dissolved in aq NaOH to liberate the free base, which was extracted with DCM (50 ml, 2x), washed with brine, collected and dried upon evaporation to get 1-(4-aminophenyl)-1-phenylethanol (**$27_{f.b}$**, 100 mg, 0.47 mmol). The final step involved adding indole-2-carbonyl chloride (**4**, 0.20 g, 1.11 mmol) to 1-(4-aminophenyl)-1-phenylethanol (**$27_{f.b}$**, 100 mg, 0.47 mmol) in 20 ml DCM and TEA (7 mmol). The mixture was refluxed for 2 days at 80 °C, with stirring. The reaction was cooled down and left to precipitate, yielding a mixture of the target compound and other side products. The reaction failed to give an isolable amount for analysis and biological screening. The chromatographic techniques failed to separate the product in a reasonable amount. R_f: 0.38 (cyclohexane: ethylacetate, 7:3).

Procedure B: To a stirred solution of phenylmagnesium chloride (**GR,** 1.66 ml, 1.69 g, 12.4 mmol) in 30 ml dry ether on an ice bath. N-(4-acetylphenyl)-1*H*-indole-2-carboxamide (**28**, 0.45 g, 1.6 mmol) in 25 mL of dry ether was added slowly, over 20 minutes at 0 °C. The reaction mixture was stirred for 1h at 0 °C, then for 12 hrs at RT, followed by quenching with 20 mL of cooled 1N HCl. The ether layer was separated, dried over anhydrous $MgSO_4$ and concentrated under reduced pressure to yield a crude paste. The paste was dissolved in 20 mL of acetone and was acidified to a pH of 2.0–3.0 by adding drops of conc HCl. The precipitated product was filtered and recrystallized with chloroform/methanol to give a pure solid (**36**, 0.22 g, 38%); R_f: 0.38 (cyclohexane: ethylacetate, 7:3); m.p.: 255 °C (decomposed); ^{1}H-NMR (300 MHz, DMSO-d_6): rotamers δ = 11.90 (br s, 1H, NH-indole), 10.65 (br s, 1H, NHCO), 8.10-6.40 (9m, 15H, Ar-H): [(8.07-7.91, m; 7.82, dd, *J*= 6.9, 8.6 Hz; 7.69, d, *J*= 7.9 Hz; s, 7.55; 7.49, d, *J*= 8.2 Hz; 7.25, dd, *J*= 7.9, 8.67.2 Hz; 7.1, dd, *J*= 7.8, 7.1 Hz; 6.96, d, *J*= 8.5 Hz; 6.62, br s, OH)], 2.51 (s, 3H, CH_3); ^{13}C-NMR (DMSO-d_6): rotamers δ = 197.04 + 196.22 (C-OH), 160.46 (CO-amide), 144.00, 137.49, 132.30, 131.56, 130.70 (CH-Ar), 129.82 (CH-Ar), 127.41, 124.56 (CH-Ar), 122.35 (CH-Ar), 120.49 (CH-Ar), 119.69 (CH-Ar), 117.30 (CH-Ar), 112.90 (CH-Ar), 105.43 (CH-Ar), 26.91 + 26.66 (CH3) ppm; Anal. Calcd for $C_{23}H_{20}N_2O_2$: C, 77.51; H, 5.66; N, 7.86. Found: C, 77.78; H, 5.52; N, 8.02.

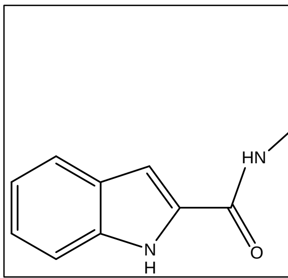
Synthesis of N-{4-[–N -(4- chlorophenyl) ethanimidoyl] phenyl}-1*H*-indole-2-carboxamide (38):



The preparation of Schiff base, was carried out by stirring a mixture of (para -acetylphenyl)-1*H*-indole-2-carboxamide (**28**, 0.40 g, 1.44 mmol) with p-chloro-aniline (**37**, 0.45 g, 3.53 mmol) in absolute methanol. The suspension was heated until clear solution was obtained . Then few drops of concentrated HCl were added as catalyst. The solution was refluxed for 2- 4 hrs at 75 °C. The precipitated product was formed during refluxing. After cooling the mixture to RT, the precipitated product formed was filtered off, washed with (10 ml) methanol, then with (10 ml) diethyl ether and dried to give a brown solid (**38**, 0.20 g, 36 %); R_f: 0.45 ($CHCl_3$/ MeOH/ Acetic acid, 94: 5: 1);m.p.: 283 °C (decomposed); ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 11.95 (br s, 1H, NH-indole), 10.75 (br s, 1H, NHCO), 8.01 (m, 1H, Ar-H), 7.92-6.75 (m, 9H, Ar-H), 2.41 (s, 3H, CH_3) ; Anal. Calcd for $C_{23}H_{18}ClN_3O$: C, 71.22; H, 4.68; N, 10.83. Found: C, 70.98; H, 4.56; N, 10.62.

6.7. Synthesis of unsubstituted and substituted indole-benzene carboxamides group (Series D):

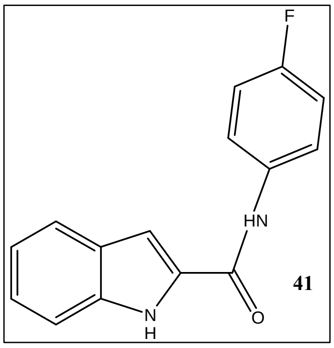
Synthesis of N-(4-chlorophenyl)-1*H*-indole-2-carboxamide (39):



39

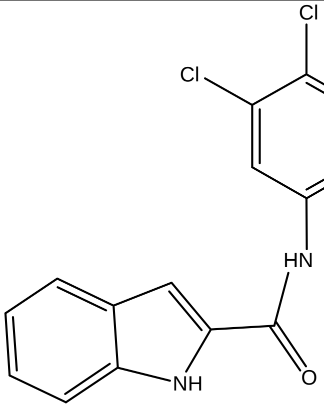
A mixture of indole-2-carbonyl chloride (**4**, 0.40 g, 2.23 mmol) and DCM (15 ml) was added dropwise to a mixture of p-chloro-aniline (**37**, 0.41 g, 3.22 mmol) and TEA (1.52 g, 15.00 mmol). The mixture was stirred at RT for 3 hrs, diluted with 15 mL of 1 N HCl, and the layers separated. The organic layer was washed with 15 mL of 2 N NaOH, washed with 15 mL of brine, and then dried over $MgSO_4$. The solvent was removed under reduced pressure recrystallized from MeOH/$CHCl_3$ to afford an off-white solid (**39**, 0.36 g, 60%); R_f: 0.60 (cyclohexane/ethylacetate, 6:4); m.p.: 259°C; ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 11.79 (br s, 1H, NH-indole), 10.35 (s, 1H, NHCO), 7.86 (d, *J*= 9.0 Hz, 2H, Ar-H), 7.69 (d, *J*= 8.1 Hz, 1H, Ar-H), 7.49 (s, 1H, Ar-H), 7.47-7.39 (m, 3H, Ar-H), 7.23 (dd, *J*= 8.1, 7.2 Hz, 1H, Ar-H), 7.08 (dd, *J*= 7.2, 7.8 Hz, 1H, Ar-H); ^{13}C-NMR (DMSO-d_6): δ = 160.26 (CONH), 138.43, 137.36, 131.66, 129.09 (C-3', 5'), 127.57, 127.44, 124.37 (CH-Ar), 122.26 (CH-Ar), 122.05 (C-2', 6'), 120.44 (CH-Ar), 112.88 (CH-Ar), 104.60 (CH-Ar) ppm; IR (thin film): ν = 3406 (NH-indole), 3318 (NHCO), 1651 (CO), 1593, 1539, 1493, 1400, 1315, 1238, 1192, 1099, 1015, 937, 826, 748 cm^{-1}; Anal. Calcd for $C_{15}H_{11}ClN_2O$: C, 66.55; H, 4.10; N, 10.35. Found: C, 66.40; H, 3.92; N, 10.12.

Synthesis of N-(4-florophenyl)-1*H*-indole-2-carboxamide (41):



A mixture of indole-2-carbonyl chloride (**4**, 0.38 g, 2.12 mmol) and DCM (15 ml) was added dropwise to a mixture of p-floro-aniline (**40**, 0.35 g, 2.12 mmol) and TEA (1.52 g, 15.00 mmol). The mixture was stirred at RT for 3 hrs, diluted with 15 mL of 1 N HCl, and the layers separated. The organic layer was washed with 15 mL of 2 N NaOH, washed with 15 mL of brine, and then dried over $MgSO_4$. The solvent was removed under reduced pressure recrystallized from MeOH/$CHCl_3$ to afford a faint yellow solid (**41**, 0.25g, 46%); R_f: 0.54 (cyclohexane/ethylacetate, 7:3); m.p.: 245°C (decomposed); ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 11.78 (br s, 1H, NH-indole), 10.80 (s, 1H, NHCO), 7.89-7.79 (m, 2H, Ar-H), 7.67 (d, *J*= 7.8 Hz, 1H, Ar-H), 7.48 (d, *J*= 8.0 Hz, 1H, Ar-H), 7.44-7.39 (m, 1H, Ar-H), 7.28-7.15 (m, 3H, Ar-H), 7.11-7.01 (m, 1H, Ar-H); ^{13}C-NMR (DMSO-d_6): rotamers δ = 160.28, 157.09 (d, $^1J_{C\text{-}F}$ = 283 Hz, C-4', CH-Ar), 160.14 (CONH), 157.09, 137.30, 135.82, 131.82, 127.47, 124.25 (CH-Ar), 122.44, 122.19 (d, $^2J_{C\text{-}F}$ = 19 Hz, C-3', 5', CH-Ar), 122.34 (CH-Ar), 120.38 (CH-Ar), 115.89, 115.59 (d, $^3J_{C\text{-}F}$ = 22.13 Hz, C-2', 6', CH-Ar), 112.86 (CH-Ar), 104.40 (CH-Ar) ppm; IR (thin film): ν = 3422 (NH-indole), 3337 (NHCO), 3271, 1639 (CO), 1543, 1508, 1408, 1339, 1315, 1223, 1188, 1096, 833, 818, 748 cm^{-1}; HRMS (ESI, negative mode): m/z (M^+ - H^+) 253.07826 ($C_{15}H_{10}FN_2O$) requires 253.07772; Anal. Calcd for $C_{15}H_{11}FN_2O$: C, 70.86; H, 4.36; N, 11.02. Found: C, 70.97; H, 4.15; N, 10.90.

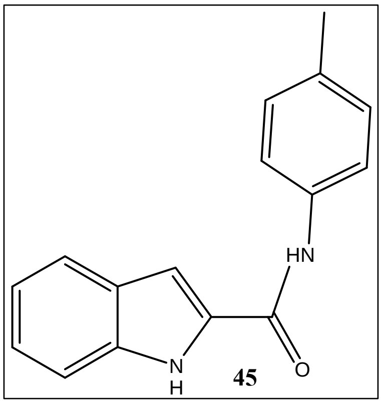
Synthesis of N-(3,4-chlorophenyl)-1*H*-indole-2-carboxamide (43):



43

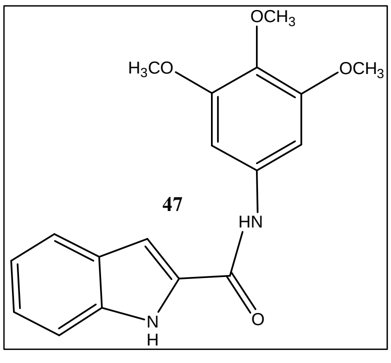
A mixture of indole-2-carbonyl chloride (**4**, 0.45 g, 2.51 mmol) and DCM (15 ml) was added dropwise to a mixture of 3,4-dichloro-aniline (**42**, 0.45 g, 2.78 mmol) and TEA (1.52 g, 15.00 mmol). The mixture was stirred at RT for 3 hrs, diluted with 15 mL of 1 N HCl, and the layers separated. The organic layer was washed with 15 mL of 2 N NaOH, washed with 15 mL of brine, and then dried over $MgSO_4$. The solvent was removed under reduced pressure recrystallized from $MeOH/CHCl_3$ to afford an off-white solid (**43**, 0.53g, 69%); R_f: 0.60 (cyclohexane/ethylacetate, 7:3); m.p.: 268°C (decomposed); ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 11.80 (br s, 1H, NH-indole), 10.45 (s, 1H, NHCO), 8.19 (s, 1H, Ar-H), 7.81 (dd, *J*= 8.8, 2.2 Hz, 1H, Ar-H), 7.70 (d, *J*= 8.0 Hz, 1H, Ar-H), 7.64 (dd, *J*= 8.2, 1.1 Hz, 1H, Ar-H), 7.51-7.41 (m, 2H, Ar-H), 7.25 (dd, *J*= 8.16, 7.02 Hz, 1H, Ar-H), 7.08 (dd, *J*= 7.05, 7.09 Hz, 1H, Ar-H); ^{13}C-NMR (DMSO-d_6): δ = 160.42 (CONH), 139.64, 137.48, 131.39, 131.28, 131.12 (CH-Ar), 127.38, 125.36, 124.58 (CH-Ar), 122.36 (CH-Ar), 121.57 (CH-Ar), 120.53 (CH-Ar), 120.42 (CH-Ar), 112.92 (CH-Ar), 104.97 (CH-Ar) ppm; IR (thin film): ν = 3395 (NH-indole), 3318 (NHCO), 1655 (CO), 1585, 1528, 1508, 1474, 1447, 1423, 1393, 1342, 1312, 1234, 1196, 1146, 1026, 810, 752, 733 cm^{-1}; HRMS (ESI, negative mode): m/z (M^+ - H^+) Anal. Calcd for $C_{15}H_{10}Cl_2N_2O$: C, 59.04; H, 3.30; N, 9.18. Found: C, 59.23; H, 3.10; N, 9.36.

Synthesis of N-(4-methylphenyl)-1*H*-indole-2-carboxamide (45):



Indole-2-carbonyl chloride (**4**, 0.40 g, 2.23 mmol) was dissolved in dioxane (15 mL) and added to a solution of p-toluidine (**44**, 0.40 g, 3.73 mmol) in hot toluene (20 mL). The mixtures were heated under reflux at 70 °C, for 2 hrs. The solvent was removed under reduced pressure and the solid was recrystallized from MeOH/$CHCl_3$ to afford an off-white solid (**45**, 0.30 g, 54%); R_f: 0.59 ($CHCl_3$/MeOH, 90:10); m.p.: 229°C; ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 11.85 (br s, 1H, NH-indole), 10.17 (br s, 1H, NHCO), 7.71 (s, 1H, Ar-H), 7.66 (d, *J* = 6.3 Hz, 2H, Ar-H), 7.51-7.34 (m, 2H, Ar-H), 7.28-7.12 (m, 2H, Ar-H), 7.07 (dd, *J* = 8.1, 6.9 Hz, 2H, Ar-H), 2.27 (s, 3H, CH_3); ^{13}C-NMR (DMSO-d_6): δ = 160.03 (CONH), 137.22, 136.87, 132.95, 132.06, 129.56 (CH-Ar), 127.51, 124.14 (CH-Ar), 122.16 (CH-Ar), 121.01, 120.63 (CH-Ar), 120.34 (CH-Ar), 112.84 (CH-Ar), 104.11 (CH-Ar), 20.97 (CH_3) ppm; IR (thin film): ν = 3418 (NH-indole), 3333 (NHCO), 2913, 1643 (CO), 1597, 1543, 1528, 1408, 1304, 1250, 1111, 934, 814, 745 cm^{-1}; HRMS (ESI, negative mode): m/z (M^+ - H^+); Anal. Calcd for $C_{16}H_{14}N_2O$: C, 76.78; H, 5.64; N, 11.19. Found: C,76.75; H, 5.92; N, 10.95.

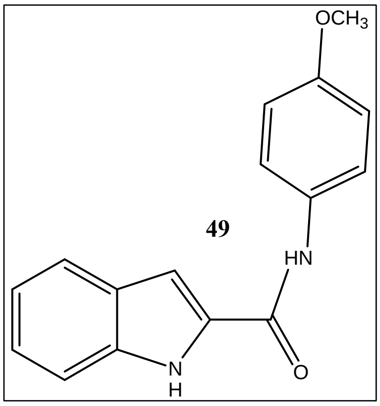
Synthesis of N-(3,4,5-trimethoxyphenyl)-1*H*-indole-2-carboxamide (47):



A mixture of indole-2-carbonyl chloride (**4**, 0.40 g, 2.23 mmol) and DCM (15 ml) was added dropwise to a mixture of 3,4,5-trimethoxy-aniline (**46**, 0.40 g, 2.18 mmol) and TEA (1.52 g, 15.00 mmol). The mixture was stirred at RT for 3 hrs, diluted with 15 mL of 1 N HCl,

and the layers separated. The organic layer was washed with 15 mL of 2 N NaOH, washed with 15 mL of brine, and then dried over $MgSO_4$. The solvent was removed under reduced pressure recrystallized from MeOH/$CHCl_3$ to afford a dark off-white solid (**47**, 0.45 g, 63%); R_f: 0.25 (cyclohexane/ethylacetate, 7:3); m.p.: 150°C; ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 11.73 (br s, 1H, NH-indole), 10.12 (br s, 1H, NHCO), 7.68 (d, *J*= 7.9 Hz, 1H, Ar-H), 7.47 (d, *J*= 8.2 Hz, 1H, Ar-H), 7.40 (br s, 1H, Ar-H), 7.28-7.19 (m, 3H, Ar-H), 7.08 (dd, *J*= 7.4, 7.4 Hz, 1H, Ar-H), 3.80 (br s, 6 H, 2 OCH_3: 3',5'-OCH_3), 3.65 (s, 3 H, 4'-OCH_3); ^{13}C-NMR (DMSO-d_6): δ = 160.04 (CONH), 153.15, 137.26, 135.53, 134.20, 132.01, 127.52, 124.24 (CH-Ar), 122.18 (CH-Ar), 120.39 (CH-Ar), 112.83 (CH-Ar), 104.10 (CH-Ar), 98.45 (CH-Ar), 60.60 (3'-OCH_3), 56.25 (5'-OCH_3), 40.91 (4'-OCH_3) ppm; IR (thin film): ν = 3333 (NH-indole), 3210 (NHCO), 2936, 2839, 1643 (CO), 1609, 1543, 1508, 1451, 1412, 1312, 1231, 1130, 999, 822, 748 cm^{-1}; HRMS (ESI, negative mode): m/z (M^+ - H^+) 325.11938 ($C_{18}H_{17}N_2O_4$) requires 325.11883; Anal. Calcd for $C_{18}H_{18}N_2O_4$: C, 66.25; H, 5.56; N, 8.58. Found: C, 65.99; H, 5.23; N, 8.35.

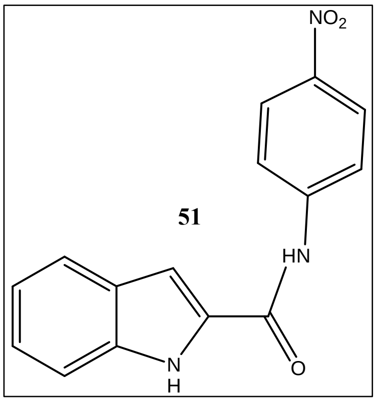
Synthesis of N-(4-methoxyphenyl) -1*H*-indole-2-carboxamide (49):



Indole-2-carbonyl chloride (**4**, 0.41 g, 2.29 mmol) was dissolved in dioxane (15 mL) and added to a solution of p-anisidine (**48**, 0.42 g, 3.41 mmol) in hot toluene (20 mL). The mixtures were heated under reflux at 80 °C, for 3 hrs. The solvent was removed under reduced pressure recrystallized from MeOH/$CHCl_3$ to afford a faint grey solid (**49**, 0.30 g, 49%); R_f: 0.79 ($CHCl_3$/MeOH, 95:5); m.p.: 133°C; ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 12.10 (br s, 1H, NH-indole), 10.48 (br s, 1H, NHCO), 7.78 (d, *J* = 6.9 Hz, 2H, H-2', 6'), 7.65 (d, *J* = 8.1 Hz, 1H, H-4), 7.49 (d, *J* = 8.7 Hz, 1H, H-7), 7.40

(s, 1H, H-3), 7.21 (dd, *J* = 8.4, 7.8 Hz, 1H, H-6), 7.05 (dd, *J* = 7.8, 6.9 Hz, 1H, H-5), 6.95 (d, *J* = 6.9 Hz, 2H, H-3', 5'), 3.75 (s, 3H, OCH_3); ^{13}C-NMR (DMSO-d_6): rotamers δ = 159.82 (CONH), 155.86 (ether), 137.23, 132.63, 132.33, 127.48, 124.0 (CH-Ar), 122.19 (CH-Ar), 122.06 (CH-Ar), 120.24 (CH-Ar), 114.25 (CH-Ar), 112.85 (CH-Ar), 104.48 (CH-Ar), 55.62 (OCH_3) ppm; IR (thin film): ν = 3399 (NH-indole), 3345 (NHCO), 3048, 2951, 2850, 1651 (CO), 1601, 1508, 1412, 1304, 1238, 1184, 1030, 826, 748 cm^{-1}; HRMS (ESI, negative mode): m/z (M^+ - H^+); Anal. Calcd for $C_{16}H_{14}N_2O_2$: C, 72.16; H, 5.30; N, 10.52. Found: C,72.24; H, 5.14; N, 10.44.

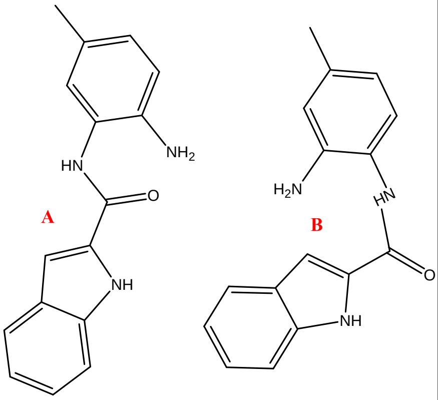
Synthesis of N-(4-nitrophenyl)-1*H*-indole-2-carboxamide (51):



A mixture of indole-2-carbonyl chloride (**4**, 0.45 g, 2.51 mmol) and DCM (15 ml) was added dropwise to a mixture of p-nitro-aniline (**50**, 0.45 g, 3.26 mmol) and TEA (1.52 g, 15.00 mmol). The mixture was stirred at RT for 3 hrs, diluted with 15 mL of 1 N HCl, and the layers separated. The organic layer was washed with 15 mL of 2 N NaOH, washed with 15 mL of brine, and then dried over $MgSO_4$. The solvent was removed under reduced pressure recrystallized from MeOH/$CHCl_3$ to afford a green-brown solid (**51**, 0.50g, 71%); R_f: 0.47 ($CHCl_3$/ MeOH/ acetic acid, 94:5:1); m.p.: □290 °C (decomposed); ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 11.84 (br s, 1H, NH-indole), 10.74 (br s, 1H, NHCO), 8.28 (d, *J*= 9.2 Hz, 2H, H-3', 5' Ar-H), 8.11 (d, *J*= 9.2 Hz, 2H, H-2', 6' Ar-H), 7.71 (d, *J*= 8.0 Hz, 1H, Ar-H), 7.58-7.45 (m, 2H, Ar-H), 7.76 (dd, *J*= 7.4, 7.5 Hz, 1H, Ar-H), 7.09 (dd, *J*= 7.6, 7.3 Hz, 1H, Ar-H); ^{13}C-NMR (DMSO-d_6): δ = 160.67 (CONH), 145.84, 142.79, 137.68, 131.15, 127.38, 125.31 (CH-Ar), 124.80 (CH-Ar), 122.45 (CH-Ar), 120.92 (CH-Ar), 120.02 (CH-Ar), 112.97 (CH-

Ar), 105.69 (CH-Ar), ppm; HRMS (ESI, negative mode): m/z (M^+ - H^+) ; Anal. Calcd for $C_{15}H_{11}N_3O_3$: C, 64.05; H, 3.94; N, 14.94. Found: C, 63.97; H, 3.89; N, 14.78.

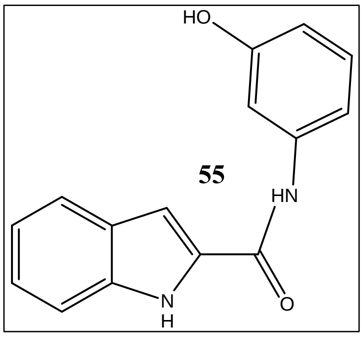
Synthesis of N-(2-amino-methylphenyl)-1*H*-indole-2-carboxamide (53):



53

A mixture of indole-2-carbonyl chloride (**4**, 0.36 g, 2.00 mmol) and DCM (15 ml) was added dropwise to a mixture of 3,4-diamino-toluene (**52**, 0.35 g, 2.87 mmol) and TEA (1.52 g, 15.00 mmol). The mixture was stirred at RT for 3 hrs, diluted with 15 mL of 1 N HCl. Then was washed with 15 mL of 2 N NaOH, washed with 15 mL of brine, and then dried over $MgSO_4$. The solvent was removed under reduced pressure recrystallized from MeOH/$CHCl_3$ to afford a pale brown solid [**53** (A or B), 0.185 g, 23%]; R_f: 0.27 (cyclohexane/ethylacetate, 7:3); m.p.: 295°C (decomposed); ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 11.85 (br s, 1H, NH-indole), 10.17 (br s, 1H, NHCO), 7.66 (d, *J*= 8.1 Hz, 1H, Ar-H), 7.63-7.53 (m, 2H, Ar-H), 7.50 (d, *J*= 8.2 Hz, 1H, Ar-H), 7.34 (s, 1H, Ar-H), 7.21 (dd, *J*= 8.2, 4.0 Hz, 1H, Ar-H), 7.14 (d, *J*= 8.4 Hz, 1H, Ar-H), 7.07 (dd, *J*= 7.3, 7.7 Hz, 1H, Ar-H), 3.47 (br s, 2H, NH_2), 2.38 (s, 3H, CH_3); ^{13}C-NMR (DMSO-d_6) rotamers: δ = 160.44, 160.38 (CONH), 137.42, 137.39, 135.36, 131.59, 131.08, 128.62, 127.50 (CH-Ar), 127.13, 126.64 (CH-Ar), 126.29 (CH-Ar), 126.00 (CH-Ar), 124.39 (CH-Ar), 124.36, 122.21 (CH-Ar), 120.51 (CH-Ar), 112.95 (CH-Ar), 104.19 + 104.12 (CH-Ar), 21.11 (CH_3) ppm; HRMS (ESI, negative mode): m/z (M^+ - H^+) 264.11424 ($C_{16}H_{14}N_3O$) requires 264.11369; Anal. Calcd for $C_{16}H_{15}N_3O$: C, 72.43; H, 5.70; N,15.84. Found: C, 72.59; H, 5.64; N, 15.98.

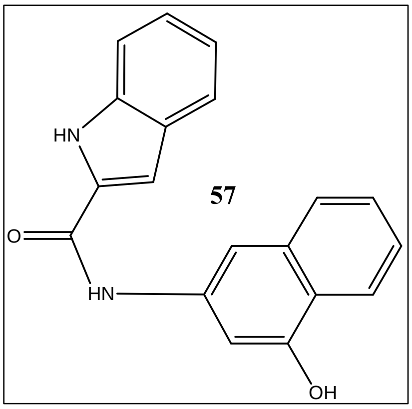
Synthesis of N-(3-hydroxyphenyl)-1H-indole-2-carboxamide (55):



55

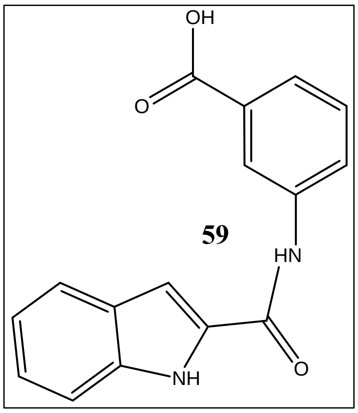
Indole-2-carbonyl chloride (**4**, 0.45 g, 2.51 mmol) was dissolved in 1,4-dioxane (15 mL) and added to a solution of 3-aminophenol (**54**, 0.45 g, 4.13 mmol) in hot toluene (50 mL). The mixtures were heated under reflux at 80 °C, for 12 hrs. The solvent was removed under reduced pressure and the residue was recrystallized from MeOH/$CHCl_3$ to afford a green-brown solid (**55**, 0.61g, 96%); R_f: 0.27 (cyclohexane/ethylacetate, 6:4); m.p.: 287 °C (decomposed); ^{1}H-NMR (300 MHz, DMSO-d_6): rotamers δ = 12.22 (br s, 1H, NH-indole), 11.84, 10.73 (2br s, 1H, NHCO) 10.52, 10.15 (2 br s, 1H, OH), 7.90 (s, 1H, Ar-H), 7.85-7.62 (m, 1H, Ar-H), 7.58-7.38 (m, 2H, Ar-H), 7.37-7.00 (m, 3H, Ar-H), 6.89-6.70 (m, 2H, Ar-H); ^{13}C-NMR (DMSO-d_6) rotamers: δ = 160.32, 158.81 (CONH), 150.78 (C-OH), 140.72, 138.36, 137.37, 133.48, 132.06, 131.68, 130.98 (CH-Ar), 130.11 (CH-Ar), 127.43, 127.21, 126.55, 125.75 (CH-Ar), 124.41 (CH-Ar), 122.81 (CH-Ar), 122.30 (CH-Ar), 120.96 (CH-Ar), 120.44 (CH-Ar), 117.91 (CH-Ar), 117.36 (CH-Ar), 115.31 (CH-Ar), 113.81 (CH-Ar), 113.67 (CH-Ar), 113.21 (CH-Ar), 112.88 (CH-Ar), 110.44 (CH-Ar), 110.05 (CH-Ar), 104.94 (CH-Ar) ppm; IR (thin film): ν = 3450 (OH), 3406 (NH-indole), 3314 (NHCO), 3055, 2920, 1701 + 1659 (CO), 1605, 1547, 1420, 1312, 1223, 1184, 741 cm^{-1}; Anal. Calcd for $C_{15}H_{12}N_2O_2$: C, 71.42; H, 4.79; N, 11.10. Found: C, 71.17; H, 4.44; N, 10.98.

Synthesis of N-(4-hydroxy-2-naphthyl)-1*H*-indole-2-carboxamide (57):



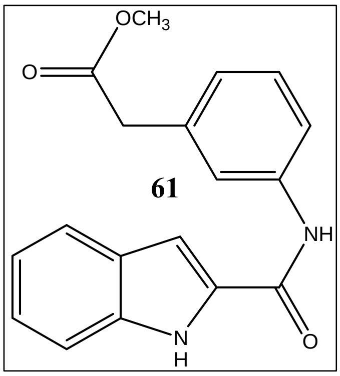
Indole-2-carbonyl chloride (**4**, 0.42 g, 2.34 mmol) was dissolved in dioxane (15 mL) and added to a solution of 3-amino-naphthol (**56**, 0.41 g, 2.58 mmol) in hot toluene (50 mL). The mixtures were heated under reflux at 80 °C, for 12 hrs. The solvent was removed under reduced pressure recrystallized from $MeOH/CHCl_3$ to afford a dark-brown solid (**57**, 0.39g, 55%); R_f: 0.18 (cyclohexane/ethylacetate, 6:4); m.p.: 296 °C (decomposed); ^{1}H-NMR (300 MHz, DMSO-d_6): rotamers δ = 12.35 (br s, 1H, NH-indole), 11.88, 11.75 (2 br s, 1H, NHCO), 10.62, 10.39 (2 br s, 1H, OH), 8.17-6.90 (m, 11H, Ar-H); ^{13}C-NMR (DMSO-d_6) rotamers: δ = 163.24, 161.15, 160.46 (CONH), 154.12, 153.92 (C-OH), 146.63, 138.55, 137.70, 137.32, 137.23, 134.29, 133.43, 131.92, 131.71, 131.17, 130.96, 129.85, 128.87, 128.06, 127.86 (CH-Ar), 127.58, 127.31, 127.07 (CH-Ar), 126.88, 126.27, 126.19 (CH-Ar), 125.90 (CH-Ar), 125.73, 125.12 (CH-Ar), 124.71 (CH-Ar), 124.50 (CH-Ar), 124.25 (CH-Ar), 124.11 (CH-Ar), 123.20, 122.89 (CH-Ar), 122.39 (CH-Ar), 122.22 (CH-Ar), 121.70 (CH-Ar), 121.30, 121.05 (CH-Ar), 120.86 (CH-Ar), 120.41 (CH-Ar), 120.01 (CH-Ar), 119.74 (CH-Ar), 119.53 (CH-Ar), 114.23 (CH-Ar), 113.27 (CH-Ar), 112.95, 112.91(CH-Ar), 112.71 (CH-Ar), 112.37, 110.44 (CH-Ar), 109.59 (CH-Ar), 108.72 (CH-Ar), 107.74 (CH-Ar), 104.71 (CH-Ar), ppm; IR (thin film): ν = 3422 (NH-indole), 3352 (NHCO), 3500-2700 (OH), 3058, 1628, 1582 + 1543 (CO), 1416, 1339, 1312, 1277, 1231, 1142, 818, 783, 748 cm^{-1}; HRMS (ESI, negative mode): m/z (M^+ - H^+) 301.09825 ($C_{19}H_{13}N_2O_2$) requires 301.09770; Anal. Calcd for $C_{19}H_{14}N_2O_2$: C, 75.48; H, 4.67; N, 9.27. Found: C, 75.18; H, 4.37; N, 8.99.

Synthesis of 3-[(1*H*-indole-2-ylcarbonyl)amino] benzoic acid (59):



Indole-2-carbonyl chloride (**4**, 0.40 g, 2.23 mmol) was added to a solution of 3-amino-benzoic acid (**58**, 0.40 g, 2.92 mmol) and TEA (1.52 g, 15.00 mmol) in 20 ml dry THF. The mixture was refluxed for 2 days at 110 °C, then cooled, washed with 1N HCl, followed by 1M sodium hydroxide and brine. The organic layer was taken, dried over $MgSO_4$ anhydrous and filtered. The organic layer was removed by evaporation under reduced pressure. The residue was purified by column chromatography using chloroform as eluent to afford a dark yellow mixture with indole (**59**, 0.100 g, 16%); R_f: 0.26 (cyclohexane/ ethylacetate/ acetic acid 3drops, 7:3); m.p.: 171°C; ^{13}C-NMR (DMSO-d_6) peaks from mixture: δ = 167.67 (COOH), 160.33 (CONH), 139.72, 137.36, 131.74, 131.66, 129.62, 129.46, 127.48, 126.23, 124.38, 122.29, 121.27, 120.41 (CH-Ar), 112.92 (CH-Ar), 107.70 (CH-Ar), 104.63 ppm; IR (thin film): ν = 3429 (NH-indole), 3414 (OH), 3306 (NHCO), 3051, 1678 (br CO), 1543, 1443, 1412, 1312, 1258, 1200, 1150, 1099, 037, 829, 748 cm^{-1}.

Synthesis of methyl-{3-[(1*H*-indole-2-yl-carbonyl)-amino] phenylacetate (61):



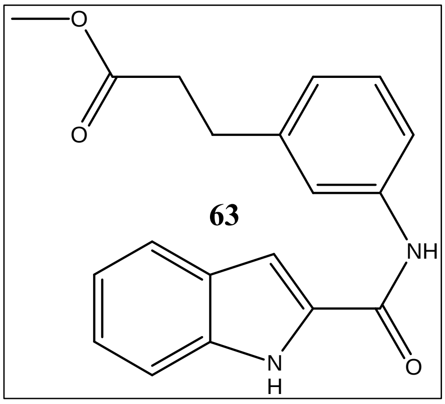
Meta-amino-phenyl acetic acid (**60,** 0.50 g, 3.31 mmol) was dissolved in 20 ml methanol and cooled in ice bath to 0 °C. Thionyl chloride (5 ml) was added drop wise and stirred at RT for 20 min then refluxed at 80 °C for 24 hrs. The residue was evaporated, washed with benzene to have 3-amino-phenyl acetic acid methyl ester (**60p**, 0.45 g, 82%). Then indole-2-carbonyl chloride (**4**, 0.40 g, 2.23 mmol) was added to 3-amino-phenyl

acetic acid methyl ester (**60p**, 0.40 g, 2.41 mmol) in 10 ml DMF in the presence of TEA (2.0 ml, 7.5 mmol). The mixture was refluxed for 2 days at 120 °C, then cooled and extracted with $CHCl_3$. The organic layer was washed with 1N HCl, followed by 1M sodium hydroxide and brine. The organic layer was taken, dried over $MgSO_4$ anhydrous and filtered. The organic layer was removed by evaporation under reduced pressure and the residue was purified by column chromatography using chloroform / methanol (95:5) as eluent to afford a yellow solid, which was the indole ester side product (**4e**, methyl indole-2-carboxylate).

The product (**61**) was minor in the sample by TLC, which could not be separated; ^{1}H-NMR (300 MHz, DMSO-d_6), peaks picked in mixture: δ = 11.91 (br s, 1H, NH-indole), 11.65 (br s, 1H, NHCO), 7.85-7.02 (m, Ar-H), 4.15 (s, $COOCH_3$), 3.62 (CH_2); ^{13}C-NMR (DMSO-d_6): δ = 167.42 ($COOCH_3$), 163.57 (CONH), 137.55, 132.22, 131.99, 130.06, 129.11, 127.47, 124.74, 124.34, 122.22, 120.22, 112.88, 107.11, 67.90 ($COOCH_3$), 38.58 (CH_2) ppm.

Data for (**4e**, methyl indole-2-carboxylate, major □ 70% of 90mg, 13%); m.p.: 205 °C; ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 11.90 (br s, 1H, NH-indole), 7.65 (d, *J* = 7.8 Hz, 1H, H-7), 7.47 (d, *J* = 8.1 Hz, 1H, H-4), 7.23 (dd, *J* = 7.8, 6.0 Hz, 1H, H-6), 7.16 (s, 1H, H-3), 7.07 (dd, *J* = 7.5, 6.2 Hz, 1H, H-5), 3.88 (s, 3H, $COOCH_3$) ppm; ^{13}C-NMR (DMSO-d_6): δ = 162.21 (*C*$OOCH_3$), 137.89, 127.54, 126.83, 125.09 (CH-Ar), 122.51 (CH-Ar), 120.63 (CH-Ar), 113.05 (CH-Ar), 108.23 (CH-Ar), 52.18 ($COOCH_3$) ppm; IR (thin film): ν = 3449 (NH), 3337, 2986, 1647 (CO), 1578 (CO-ester), 1543, 1420, 1335, 1261, 1150, 1107, 779, 748 cm^{-1}.

Synthesis of methyl 3-{3-[(1*H*-indole-2-ylcarbonyl)-amino] phenylpropanoate (63):

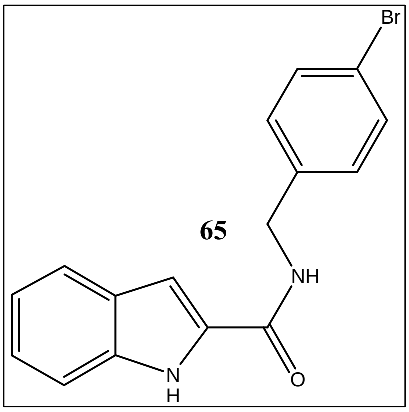


Meta-amino-phenyl propionic acid (**62,** 0.50 g, 3.03 mmol) was dissolved in 20 ml methanol and cooled in ice bath to 0 °C, a 5 ml $SOCl_2$ was added drop wise and stirred at RT for 20 min then refluxed at 80 °C for 24 hrs, the residue was evaporated, washed with benzene to have 3-amino-phenyl propionic acid methyl ester (**62p,** 0.42 g, 78%). Then indole-2-carbonyl chloride (**4**, 0.40 g, 2.23 mmol) was added to 3-amino-phenyl propionic acid methyl ester (**62p**, 0.40 g, 2.25 mmol) in 10 ml DMF in the presence of TEA (2.0 ml, 7.5 mmol). The mixture was refluxed for 2 days at 120 °C, then cooled and extracted with $CHCl_3$. The organic layer was washed with 1N HCl, followed by 1M sodium hydroxide and brine. The organic layer was taken, dried over $MgSO_4$ anhydrous and filtered. The organic layer was removed by evaporation under reduced pressure and the residue was purified by column chromatography using chloroform / methanol (95:5) as eluent to afford a yellow mixture with indole acyl (90 mg, 13%); R_f: 0.29 (cyclohexane/ ethylacetate/ acetic acid 3drops, 7:3); m.p.: 215 °C; which was proven by NMR to be the indole-2-carboxylic acid (**3**). The product was minor that could not be separated.

Data for indole 2-carboxylic acid: ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 11.65 (br s, 1H, NH-indole), 7.61 (d, *J* = 7.8 Hz, 1H, Ar-H), 7.44 (d, *J* = 8.1 Hz, 1H, Ar-H), 7.20 (dd, *J* = 7.8, 7.2 Hz, 1H, Ar-H), 7.10-6.94 (m, 2H, Ar-H); ^{13}C-NMR (DMSO-d_6): δ = 164.20 (CO), 138.93, 129.35, 127.81, 123.84, 122.02, 119.97, 112.82, 106.14 ppm; IR (thin film): ν = 3449 (NH-indole), 3422, 3059, 2812, 1574 (CO), 1543, 1420, 1362, 1331, 1231, 1103, 1015, 926, 791, 733 cm^{-1}.

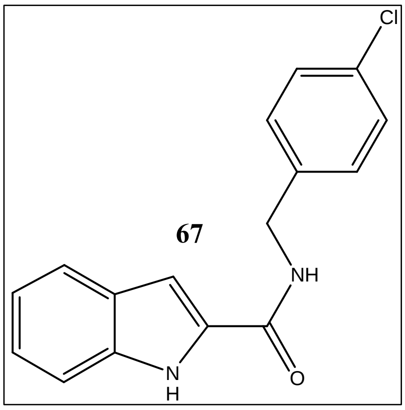
6.8. Synthesis of unsubstituted indole benzyl and phenyl-ethyl carboxamides group (Series E):

Synthesis of N-(4-bromobenzyl)-1*H*-indole-2-carboxamide (65):



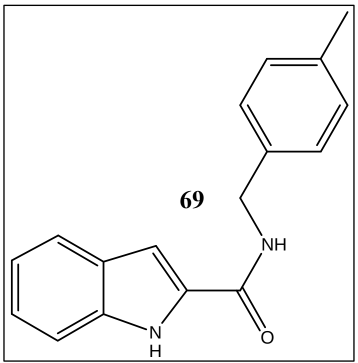
A mixture of indole-2-carbonyl chloride (**4**, 0.40 g, 2.23 mmol) in 25 ml $CHCl_3$ and 4-bromo-benzylamine (**64**, 0.40 g, 2.15 mmol) was refluxed for 3 hrs at 80 °C, then cooled to RT and worked up. The separated solid was filtered, washed with ethanol, dried and crystallized from chloroform to give a white solid (**65**, 0.570 g, 81%); R_f: 0.40 (cyclohexane/ ethylacetate, 7:3); m.p.: 250 °C; ^{1}H-NMR (300 MHz, DMSO-d_6) : rotamers δ = 11.65 (br s, 1H, NH-indole), 9.18 (t, *J* = 5.7 Hz, 1H, NHCO), 8.55-7.00 (6m, 9H, Ar-H): [(8.54, br s; 7.62, d, *J*= 8.4 Hz; 7.55-7.40, m, *J* = 8.4, 8.4, 8.3 Hz; 7.31, d, *J* = 8.1 Hz; 7.22-7.13, m; 7.03, dd, *J* = 7.8, 6.9 Hz)], 4.48-3.99 (2H, CH_2): [(4.48 d, *J*= 6.0 Hz; 3.99, s)]; ^{13}C-NMR (DMSO-d_6): rotamers δ = 161.62 (CONH), 139.64, 136.95, 133.99, 131.97, 131.89 (CH-Ar), 131.73 (CH-Ar), 131.62 (CH-Ar), 129.94 (CH-Ar), 127.52, 123.80 (CH-Ar), 122.18, 121.96 (CH-Ar), 120.23, 120.19 (CH-Ar), 112.76 (CH-Ar), 103.31 (CH-Ar), 42.04 + 41.93 (CH_2) ppm; IR (thin film): ν = 3414 (NH-indole), 3283 (NHCO), 3001, 2947, 2627, 1636 (CO), 1547, 1489, 1420, 1381, 1312, 1254, 1072, 1011, 822, 752 cm^{-1}; Anal. Calcd for $C_{16}H_{13}BrN_2O$: C, 58.38; H, 3.98; N, 8.51. Found: C, 58.15; H, 3.85; N, 8.33.

Synthesis of N(4-chlorobenzyl)-1*H*-indole-2-carboxamide (67):



To a solution of indole-2-carbonyl chloride (**4**, 0.41 g, 2.29 mmol) in 25 ml $CHCl_3$, 4-chloro-benzylamine (**66**, 0.41 g, 2.90 mmol) was added and the reaction mixture was refluxed for 3 hrs at 80 °C. The mixture was cooled to RT and the separated solid was filtered, washed with ethanol, dried and crystallized from chloroform to give a white solid (**67**, 0.405 g, 62%); R_f: 0.40 (cyclohexane/ ethylacetate, 7:3); m.p.: 225 °C; ^{1}H-NMR (300 MHz, DMSO-d_6): rotamers δ = 11.65 (br s, 1H, NH-indole), 9.17 (t, *J* = 5.7 Hz, 1H, NHCO), 8.55-7.00 (5m, 9H, Ar-H): [(8.53, br s; 7.61, d, *J*= 8.1 Hz; 7.58-7.32, m; 7.22-7.12, m; 7.03, dd, *J* = 7.8, 6.9 Hz)], 4.49, 4.01 (2H, CH_2): [(4.49, d, *J* = 6.0 Hz; 4.01, s)]; ^{13}C-NMR (DMSO-d_6): rotamers δ = 161.62 (CONH), 139.21, 136.94, 133.60, 131.98, 131.76, 131.43 (CH-Ar), 129.56 (CH-Ar), 128.96 (CH-Ar), 128.70 (CH-Ar), 127.51, 123.80 (CH-Ar), 121.96 (CH-Ar), 120.19 (CH-Ar), 112.76 (CH-Ar), 103.30 (CH-Ar), 41.97 + 41.86 (CH_2) ppm; IR (thin film): ν = 3414 (NH-indole), 3283 (NHCO), 3102, 2909, 2886, 2685, 1636 (CO), 1597, 1547, 1520, 1493, 1412, 1381, 1312, 1250, 1215, 1096, 1018, 826, 748 cm^{-1}; Anal. Calcd for $C_{16}H_{13}ClN_2O$: C, 67.49; H, 4.60; N, 9.84. Found: C, 67.14; H, 4.45; N, 9.78.

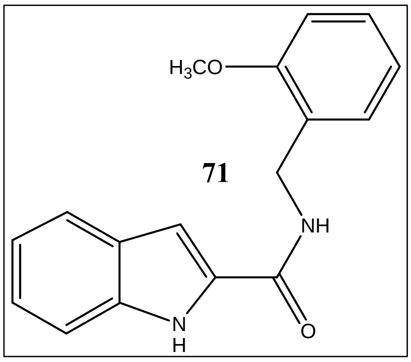
Synthesis of N(4-methylbenzyl)-1*H*-indole-2-carboxamide (69):



69

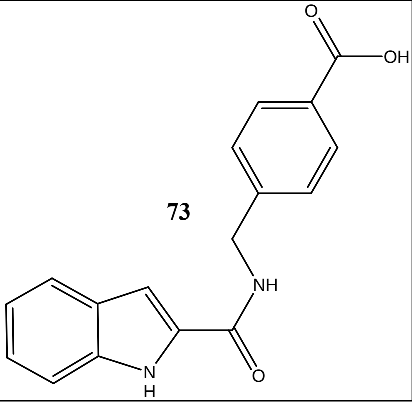
To a solution of indole-2-carbonyl chloride (**4**, 0.42 g, 2.34 mmol) in 30 ml DCM, 4-methyl-benzylamine (**68**, 0.41 g, 3.39 mmol) was added. The reaction mixture was refluxed for 2 hrs at 80 °C, then cooled to RT. The separated product was filtered, washed with ethanol, dried and crystallized from chloroform to give a white solid (**69**, 0.580 g, 94%); R_f: 0.40 (cyclohexane/ ethylacetate, 7:3); m.p.: 202 °C; ^{1}H-NMR (300 MHz, DMSO-d_6): rotamers δ = 11.65 (br s, 1H, NH-indole), 9.10 (t, *J* = 5.77 Hz, 1H, NHCO), 8.48-7.00 (5m, 9H, Ar-H): [(8.46, br s; 7.61, d, *J*= 8.0 Hz; 7.48-7.33, dd, *J* = 8.3, 7.3 Hz; 7.28-7.09, m; 7.03, dd, *J*= 7.2, 7.7 Hz)], 4.47, 3.95 (2H, CH_2): [(4.47, d, *J*= 5.8 Hz; 3.95, s)], 2.31, 2.27 (2s, 3H, CH_3); ^{13}C-NMR (DMSO-d_6): rotamers δ= 161.49 (CONH), 138.18, 137.09, 136.90, 136.25, 132.18, 131.57, 129.52 (CH-Ar), 129.38 (CH-Ar), 129.28 (CH-Ar), 127.69 (CH-Ar), 127.54 (CH-Ar), 123.71 (CH-Ar), 121.93 (CH-Ar), 120.14 (CH-Ar), 112.74 (CH-Ar), 103.17 (CH-Ar), 42.36 + 42.31 (2s, CH_2), 21.21 + 21.14 (2s, CH_3) ppm; IR (thin film): ν = 3418 (NH-indole), 3302 (NHCO), 3102, 3009, 2920, 2889, 2689, 2612, 1639 (CO), 1582, 1566, 1516, 1416, 1312, 1265, 1115, 1069, 1018, 972, 814, 745 cm^{-1}; Anal. Calcd for $C_{17}H_{16}N_2O$: C, 77.25; H, 6.10; N, 10.60. Found: C, 76.98; H, 6.34; N, 10.38.

Synthesis of N-(2-methoxybenzyl)-1*H*-indole-2-carboxamide (71):



To a solution of indole-2-carbonyl chloride (**4**, 0.38 g, 2.12 mmol) in 25 ml DCM, 2-methoxy-benzylamine (**70**, 0.35 g, 2.55 mmol) was added. The reaction mixture was refluxed for 2 hrs at 80 °C, then cooled to RT. The separated product was filtered, washed with ethanol, dried and crystallized from chloroform to give a white solid (**71**, 0.399 g, 67%); R_f: 0.31 (cyclohexane/ ethylacetate, 7:3); m.p.: 203 °C; ^{1}H-NMR (300 MHz, DMSO-d_6): rotamers δ = 11.65 (br s, 1H, NH-indole), 9.08 (t, *J* = 5.4 Hz 1H, NHCO), 8.45-6.85 (8m, 9H, Ar-H): [(8.42, br s; 7.60, d, *J* = 7.8 Hz; 7.43, d, *J*= 8.4 Hz; 7.27, d, *J* = 7.8 Hz; 7.22-7.13, m; 7.07-6.95, m; 6.96, d, *J* = 6.9 Hz; 6.89, d, *J* = 6.9 Hz)], 4.43, 3.92 (2H, CH_2): [(4.43, d, *J* = 5.7 Hz; 3.92, s)], 3.76, 3.72 (3H, OCH_3): [(3.76, d, *J* = 1.8 Hz; 3.71, d, *J* = 1.8 Hz)]; ^{13}C-NMR (DMSO-d_6): rotamers, δ = 161.43 + 159.79 + 158.67 (CONH), 136.88, 132.21, 132.08, 130.99 (CH-Ar), 129.07 (CH-Ar), 127.54, 126.46, 123.70 (CH-Ar), 121.93 (CH-Ar), 120.13 (CH-Ar), 114.36 (CH-Ar), 114.15 (CH-Ar), 112.73 (CH-Ar), 103.15 (CH-Ar), 55.64 + 55.52 (OCH_3), 42.09 + 42.01 (CH_2) ppm; IR (thin film): ν = 3414 (NH-indole), 3279 (NHCO), 2947, 2889, 2866, 1628 (CO), 1612, 1547, 1516, 1420, 1377, 1304, 1254, 1184, 1126, 1026, 945, 833, 814, 752 cm^{-1}; Anal. Calcd for $C_{17}H_{16}N_2O_2$: C, 72.84; H, 5.75; N, 9.99. Found: C, 72.69; H, 5.45; N, 9.65.

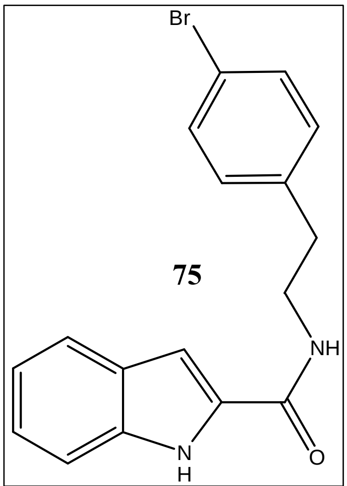
Synthesis of N-(4-amino-methyl benzoic acid)-1*H*-indole-2-carboxamide (73):



73

To a solution of indole-2-carbonyl chloride (**4**, 0.38 g, 2.12 mmol) and $NaOC_2H_5$ (0.35 g, 5.14 mmol) in 20 ml dry DCM, 4-aminomethyl benzoic acid (**72**, 0.35 g, 2.32 mmol) was added. The reaction mixture was refluxed for 2 days at 80 °C, then cooled to RT. The separated product was filtered, washed with ethanol, dried and crystallized from chloroform to give a yellow solid (**73**, 70 mg, 11%); R_f: 0.79 (cyclohexane: ethylacetate, 7:3); m.p.: 184 °C; HRMS (ESI, negative mode): m/z (M^+ - H^+) 293.09317 ($C_{17}H_{13}N_2O_3$) requires 293.09262; IR (thin film): ν = 3441 (NH-indole), 3298 (NHCO), 2982, 2947, 1659 (CO), 1639 (CO), 1589, 1524, 1404, 1315, 1281, 1238, 1200, 1177, 926, 826, 739 cm^{-1}; Anal. Calcd for $C_{17}H_{14}N_2O_3$: C, 69.38; H, 4.79; N, 9.52. Found: C, 69.39; H, 4.33; N, 9.43. NMR analysis was not obtained because of the low yield of the product and bad solubility.

Synthesis of N-[2-(4-bromophenyl)ethyl]-1*H*-indole-2-carboxamide (75):



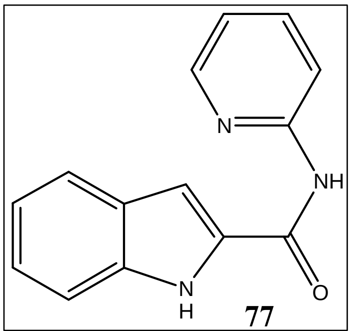
75

To a solution of indole-2-carbonyl chloride (**4**, 0.38 g, 2.12 mmol) in 25 ml DCM, 4-bromo-phenyethylamine (**74**, 0.40 g, 2.15 mmol) was added. The reaction mixture was refluxed for 2 hrs at 80 °C, then cooled to RT. The separated product was filtered, washed with ethanol, dried and crystallized from chloroform to give a white solid (**75**, 0.150 g, 25%); R_f: 0.35 (cyclohexane/ ethylacetate/ 3 dropa acetic acid, 7:3); m.p.: 223 °C; ^{1}H-NMR (300 MHz, DMSO-d$_6$): rotamers δ = 11.57 (br

s, 1H, NH-indole), 8.68 (br s, 1H, NHCO), 8.18-6.93 (4m, 9H, Ar-H): [(8.21, br s; 7.60, d, *J* = 7.8 Hz; 7.56-7.38, m; 7.29-6.97, m)], 3.50, 3.00 (2m, 2H, $NHCH_2$), 2.90 (m, 2H, CH_2-Ar); ^{13}C-NMR (DMSO-d_6): rotamers, δ = 161.50 (CONH), 139.73, 137.41, 136.82, 132.22, 131.88 (CH-Ar), 131.48 (CH-Ar), 127.43, 123.62 (CH-Ar), 121.90 (CH-Ar), 120.15 (CH-Ar), 120.09, 112.73 (CH-Ar), 102.98 (CH-Ar), 40.03 + 40.25 ($NHCH_2$), 32.69 +34.93 (CH_2-Ar) ppm; IR (thin film): ν = 3441 (NH-indole), 3244 (NHCO), 2994, 2982, 2897, 2797, 2727, 2612, 2546, 2415, 1647 (CO), 1601, 1485, 1408, 1258, 1072, 1015, 829, 802 cm^{-1}; Anal. Calcd for $C_{17}H_{15}BrN_2O$: C, 59.49; H, 4.41; N, 8.16. Found: C, 59.21; H, 4.63; N, 7.98.

6.9. Miscellaneous: unsubstituted indole heterocyclic carboxamides group (Series F):

Synthesis of N-(Pyridin-2-yl)-1*H*-indole-2-carboxamide (77):

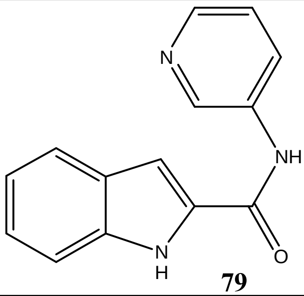


77

Indole-2-carbonyl chloride (**4**, 0.42 g, 2.34 mmol) was added to 2- aminopyridine (**76**, 0.42 g, 4.51 mmol) in 25 ml DMF in the presence of TEA (2.0 ml, 7.5 mmol). The mixture of reaction was then refluxed for 2 days at 150 °C. DMF was removed by evaporation under reduced pressure and the residue was redissolved in $CHCl_3$ and extracted in acidic water. The organic layer, after drying over anhydrous $MgSO_4$, was purified by column chromatography using $CHCl_3$ as eluent to afford the title compound as a faint yellow solid (**77**, 180 mg, 32%); R_f: 0.53 ($CHCl_3$/ MeOH/ acetic acid, 94:5:1); m.p.: 260 °C (decomposed); ^{1}H-NMR (300 MHz, DMSO-d_6): rotamers, δ = 13.75 (br s, 1H, NH-indole), 11.8 + 10.91 (2br s, 1 H, NHCO), 8.61-7.78 (m, 3H, Ar-H), 7.75-7.35 (m, 3H, Ar-H), 7.30-6.80 (m, 3H, Ar-H) ppm; ^{13}C-NMR (DMSO-d_6): δ = 160.11 (CONH), 144.10, 137.60, 137.24,

133.32, 127.56, 124.76, 113.56, 112.45, 111.90, 108.37, 104.99 ppm; IR (thin film): ν = 3449 (NH-indole), 3422 (NHCO), 3144, 2924, 1701 (CO), 1558, 1450, 1366, 1346, 1200, 1080, 748 cm^{-1}; HRMS (ESI, negative mode): m/z (M^+ - H^+) 236.08294 ($C_{14}H_{11}N_3O$) requires 236.08239; Anal. Calcd for $C_{14}H_{11}N_3O$: C, 70.87; H, 4.67; N, 17.71. Found: C, 70.62; H, 4.88; N, 17.55.

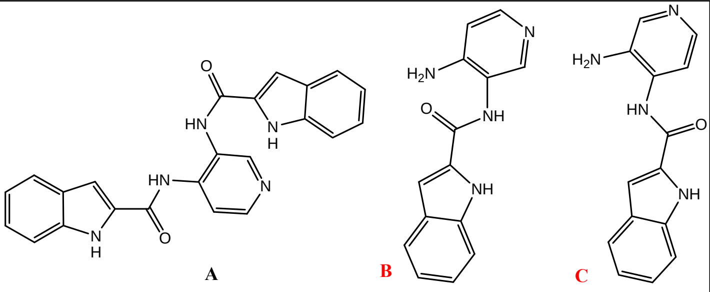
Synthesis of N-(Pyridin-3-yl)-1*H*-indole-2-carboxamide (79):



79

Indole-2-carbonyl chloride (**4**, 0.42 g, 2.34 mmol) was added to 3- aminopyridine (**78**, 0.42 g, 4.51 mmol) in 25 ml DMF in the presence of TEA (2.0 ml, 7.5 mmol). The mixture of reaction was then refluxed for 2 days at 150 °C. DMF was removed by evaporation under reduced pressure and the residue was redissolved in $CHCl_3$ and extracted in acidic water. The organic layer, after drying over anhydrous $MgSO_4$, was purified by column chromatography using $CHCl_3$ as eluent to afford the title compound as a faint yellow solid (**79**, 180 mg, 32%); R_f: 0.21 ($CHCl_3$/ MeOH/ acetic acid, 94:5:1); m.p.: 250 °C (decomposed); ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 11.90 (br s, 1H, NH-indole), 10.51 (br s, 1 H, NHCO), 9.02 (s, 1H, Ar-H), 8.33 (d, *J* = 4.19 Hz, 1H, Ar-H), 8.25 (d, *J* = 8.12 Hz, 1H, Ar-H), 7.70 (d, *J* = 7.87 Hz, 1H, Ar-H), 7.53-7.32 (m, 3H, Ar-H), 7.25 (dd, *J* = 7.21, 7.70 Hz, 1H, Ar-H), 7.09 (dd, *J* = 7.46, 7.20 Hz, 1H, Ar-H); ^{13}C-NMR (DMSO-d_6): rotamers δ = 160.57 (CONH), 144.80 (CH-Ar), 142.11 (CH-Ar), 137.42, 136.21, 131.41, 127.59 (CH-Ar), 127.43, 124.49, 124.09 (CH-Ar), 122.32 (CH-Ar), 120.48 (CH-Ar), 112.90 (CH-Ar), 104.97 (CH-Ar) ppm; IR (thin film): ν = 3449 (NH-indole), 3422 (NHCO), 3144, 2924, 1701 (CO), 1558, 1450, 1366, 1346, 1200, 1080, 748 cm^{-1}; Anal. Calcd for $C_{14}H_{11}N_3O$: C, 70.87; H, 4.67; N, 17.71. Found: C, 70.67; H, 4.38; N, 17.72.

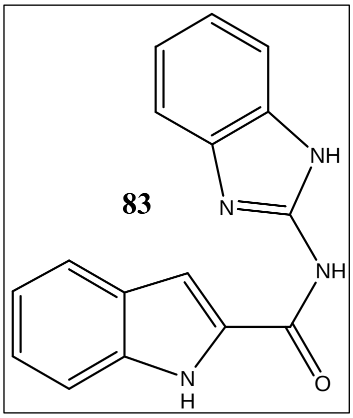
Synthesis of N-{3[(1*H*-indole-2-yl carbonyl) amino] pyridine-4-yl}-1*H*-indole-2-carboxamide (81):



81

Indole-2-carbonyl chloride (**4**, 0.42 g, 2.34 mmol) was added to 3,4-diaminopyridine (**80**, 0.42 g, 3.85 mmol) in 20 ml DMF in the presence of TEA (2.0 ml, 7.5 mmol). The mixture of reaction was then refluxed for 2 days at 150 °C. DMF was removed by evaporation under reduced pressure and the residue was redissolved in $CHCl_3$ and extracted in acidic water. The organic layer was dried over anhydrous $MgSO_4$, then purified by column chromatography using $CHCl_3$ as eluent to afford the title compound **81A** not **81B or C** as confirmed by NMR and MS analysis. The title compound is brown solid (**81 A**, 220 mg, 24%); m.p.: 255 °C; ^{1}H-NMR (300 MHz, DMSO-d$_6$): δ = 11.92 + 13.61 (2 br s, 2H, 2 NH-indole), 10.25 + 11.92 (2 br s, 2H, 2 NHCO), 8.47 (s, 2H, Ar-H), 8.40-7.90 (m, 2H, Ar-H), 7.85-7.38 (m, 3H, Ar-H), 7.23 (dd, *J*= 9.0, 6.0 Hz, 1H, Ar-H), 7.16-6.94 (m, 2H, Ar-H), 6.74 (d, *J*= 6.0 Hz, 1H, Ar-H); ^{13}C-NMR (DMSO-d$_6$): δ = 161.04 (CONH), 155.98 (CONH), 148.97, 137.80 (CH-Ar), 137.66 (CH-Ar), 137.32, 132.51, 131.18 (CH-Ar), 130.98, 127.35, 124.49 (CH-Ar), 122.28 (CH-Ar), 120.99, 120.45 (CH-Ar), 119.63, 112.86 (CH-Ar), 110.03 (CH-Ar), 106.97 (CH-Ar), 106.05 (CH-Ar) ppm; IR (thin film): ν = 3356 (NH-indole), 3198 (NHCO), 3063, 2920, 2901, 2862, 1680 (CO), 1651 (CO), 1597, 1562, 1535, 1504, 1412, 1369, 1312, 1234, 1196, 810 cm^{-1}; HRMS (ESI, positive mode): m/z (M^+ + H^+): [395 (2%); 381.29 (35%); 253 (55%)] 253.10839 ($C_{23}H_{18}N_5O_2$) requires 253.10894; Anal. Calcd for $C_{23}H_{17}N_5O_2$: C, 69.86; H, 4.33; N, 17.71. Found: C, 69.66; H, 3.93; N, 18.01.

Synthesis of N-(1*H*-Benzimidazol-2-yl)-1*H*-indole-2-carboxamide (83):

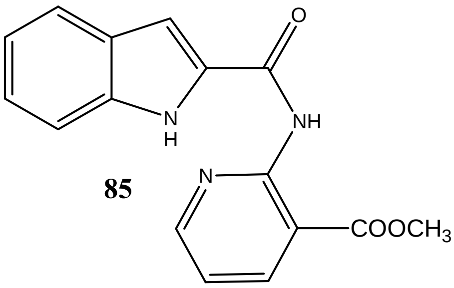


Procedure A: Indole-2-carbonyl chloride (**4**, 0.40 g, 2.23 mmol) was added to a solution of 2- aminbenzimidazole (**82**, 0.40 g, 3.01 mmol) and $NaOC_2H_5$ (0.35 g, 5.14 mmol) in 20 ml dry $CHCl_3$. The mixture was refluxed for 18 hrs at 80 °C, then cooled, washed with 1N HCl, followed by 1M sodium hydroxide and brine. The organic layer was taken, dried over $MgSO_4$ anhydrous and filtered. The organic layer was removed by evaporation under reduced pressure and the residue was purified by column chromatography using chloroform / methanol (95:5) as eluent to afford a yellow solid (**83**, 40 m g, 7%).

Procedure B: Indole-2-carbonyl chloride (**4**, 0.40 g, 2.23 mmol) was added to 2-aminobenzimidazole (**82**, 0.40 g, 3.01 mmol) in 25 ml DMF in the presence of TEA (2.0 ml, 7.5 mmol). The mixture of reaction was then refluxed for 2 days at 150 °C. DMF was removed by evaporation under reduced pressure and the residue was redissolved in $CHCl_3$ and extracted in acidic water. The organic layer, after drying over anhydrous $MgSO_4$,was purified by column chromatography using as eluent $CHCl_3$ to afford the title compound as yellow solid (**83**, 90 mg, 15%); R_f: 0.43 ($CHCl_3$/ MeOH/ acetic acid, 94:5:1); m.p.: 300 °C (decomposed); ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 12.09 (br s, 2 H, NH-indole, NH-benzimidazole), 11.73 (br s, 1H, NHCO), 7.84 (br s, 1H, Ar-H), 7.80-7.49 (m, 2H, Ar-H), 7.55 (d, *J*= 6.0 Hz, 1H, Ar-H), 7.50-7.27 (m, 3H, Ar-H), 7.27-7.00 (m, 2H, Ar-H); ^{13}C-NMR (DMSO-d_6): δ = 160.34 (CONH), 144.98, 138.31, 130.38, 129.45, 127.25, 125.77 (CH-Ar), 124.54 (CH-Ar), 122.92 (CH-Ar), 122.37, 121.01 (CH-Ar), 120.40, 114.01 (CH-Ar), 113.16 (CH-Ar), 108.40 (CH-Ar), 107.73 ppm; IR (thin film): ν = 3510 (NH-imidazole), 3422 (NH-indole), 3248

(NHCO), 3059, 1686 (CO), 1601, 1520, 1342, 1315, 1211, 1184, 1146, 1111, 741 cm^{-1}; HRMS (ESI, negative mode): m/z (M^+ - H^+) 275.09383 ($C_{16}H_{11}N_4O$) requires 275.09329; Anal. Calcd for $C_{16}H_{12}N_4O$: C, 69.55; H, 4.38; N, 20.28. Found: C, 69.85; H, 4.62; N, 19.95.

Synthesis of methyl 2-[(1*H*–indole–2–ylcarbonyl)amino] nicotinate (85):



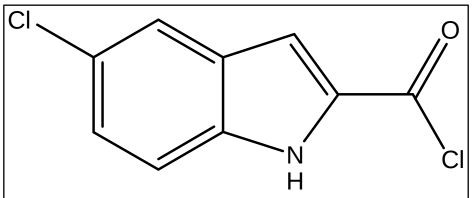
85

Ortho-amino-nicotinic acid (**84,** 0.45 g, 3.26 mmol) was dissolved in 20 ml methanol and cooled in ice bath to 0 °C. Thionyl chloride (5 ml) was added drop wise and stirred at RT for 20 minutes then refluxed at 80 °C for 12 hrs. The residue was evaporated, washed with benzene to have methyl-2-amino nicotinate (**84p,** 0.38 g, 77%). Then indole-2-carbonyl chloride (**4**, 0.40 g, 2.23 mmol) was added to methyl-2-amino nicotinate (**84p**, 0.38 g, 2.50 mmol) in 10 ml DMF in the presence of TEA (2.0 ml, 7.5 mmol). The mixture was refluxed for 2 days at 120 °C, then cooled and extracted with $CHCl_3$. The organic layer was washed with 1N HCl, followed by 1M sodium hydroxide and brine. The organic layer was taken, dried over $MgSO_4$ anhydrous, filtered and reduced by evaporation. The residue was purified by column chromatography using chloroform / methanol (95:5) as eluent to afford a yellow solid (**85**, 80 mg, 12%); R_f: 0.61 (cyclohexane: ethylacetate, 6:4); m.p.: 223 °C; ^{1}H-NMR (300 MHz, DMSO-d_6) rotomers: δ = 11.26, 11.20 (2br s, 1H, NH-indole), 8.37 (s, 1H, NHCO), 8.33 (s, 1H, Ar-H), 7.58-7.29 (m, 3H, Ar-H), 7.18-6.74 (m, 4H, Ar-H), 3.36 (s, 3H, CH_3-ester); ^{13}C-NMR (DMSO-d_6): δ= 180.40 (CO-ester), 168.11 (CH-Ar), 167.83 (CONH), 136.44, 135.92,

127.89, 122.86 (CH-Ar), 121.59 (CH-Ar), 119.48 (CH-Ar), 112.56 (CH-Ar), 104.44, 103.54, 61.36 (CO-ester) ppm; Anal. Calcd for $C_{16}H_{13}N_3O_3$: C, 65.08; H, 4.44; N, 14.23. Found: C, 65.39; H, 4.25; N, 14.64.

6.10. Substituted indole carboxamides group (Series G):

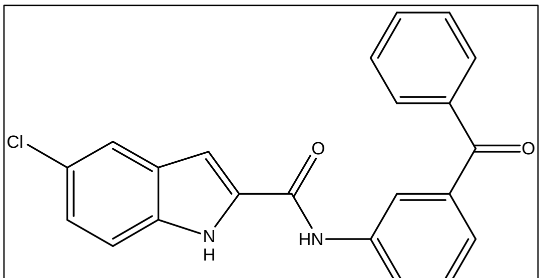
Synthesis of 5-chloroindole-2-carbonyl chloride (87) (Onda *et al*., 2008):



87

A mixture of 5-chloroindole-2-carboxylic acid (**86**, 1 g, 5.1 mmol) and $SOCl_2$ (3.6 mL, 50 mmol) in 20 mL of dry DCM was added in a round bottomed flask. The reaction mixture was refluxed for 18 hrs at 70–80 °C till the completion of the reaction. Solvent was evaporated under vacuum, then DCM was added twice and evaporated again to dryness in order to remove the excess of $SOCl_2$ present, to afford acid chloride (**87**, 0.95 g , 86%) as a yellow precipitate; R_f: 0.73 ($CHCl_3$/ MeOH/ acetic acid, 94:5:1); m.p.= 209 °C; ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 11.97 (s, 1H, NH-indole), 7.70 (s, 1H, H-4), 7.45 (d, *J* = 8.7 Hz, 1H, H-6), 7.23 (d, *J* = 8.8 Hz, 1H, H-7), 7.07 (s, 1H, H-3); ^{13}C-NMR (DMSO-d_6): δ = 162.94 (CO-Cl), 136.05, 130.41, 128.31, 124.93, 124.84 (CH-Ar), 121.41 (CH-Ar), 114.59 (CH-Ar), 107.25 (CH-Ar) ppm.

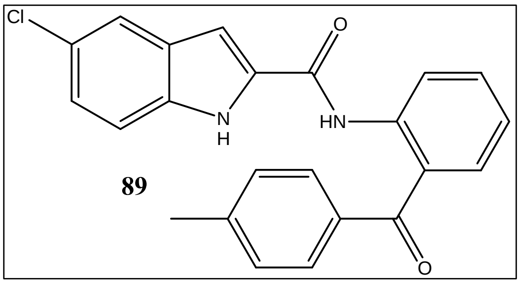
Synthesis of N-[3-benzoylphenyl]-5-chloro-1*H*-indole-2-carboxamide (88):



88

5-Chloroindole-2-carbonyl chloride (**87**, 0.41 g, 1.9 mmol) was added to a solution of 3-aminobenzophenone (**9**, 0.35 g, 1.8 mmol) in 15 ml acetonitrile and 20 ml dry toluene. The mixture was refluxed for 24 hrs at 90 °C, then cooled. The organic layer was removed by evaporation under reduced pressure and the residue was crystallized using chloroform / methanol to afford a yellow crystals (**88**, 0.31 g, 46 %); R_f: 0.37 (cyclohexane : ethylacetate, 7:3); m.p.: 255 °C; ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 12.01 (br s, 1H, NH-indole), 10.54 (s, 1H, NHCO), 8.25 (s, 1H, Ar-H), 8.18 (d, *J* = 7.97 Hz, 1H, Ar-H), 7.84 – 7.72 (m, 3H, Ar-H), 7.69 (d, *J* = 7.20 Hz, 1H, Ar-H), 7.65 – 7.53 (m, 3H, Ar-H), 7.50 (d, *J* = 4.75 Hz, 1H, Ar-H), 7.46 (br s, 2H, Ar-H), 7.24 (dd, *J* = 8.61, 8.61, 1.6 Hz, 1H, Ar-H); ^{13}C-NMR (DMSO-d_6): δ = 196.07 (CO-ketone), 160.07 (CONH), 139.52, 137.90, 137.53, 135.75, 133.16 (CH-Ar), 133.10, 130.11 (CH-Ar), 129.58 (CH-Ar), 129.05 (CH-Ar), 128.48, 125.23 (CH-Ar), 124.93, 124.51(CH-Ar), 124.43 (CH-Ar), 121.62 (CH-Ar), 121.35 (CH-Ar), 114.48 (CH-Ar), 104.18 (CH-Ar) ppm; Anal. Calcd for $C_{22}H_{15}ClN_2O_2$: C, 70.50; H, 4.03; N, 7.47. Found: C, 70.24; H, 4.10; N, 7.29.

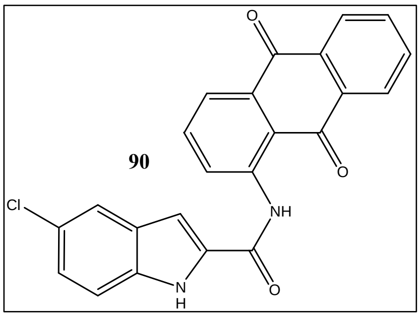
Synthesis of *N-[2-(4-toloylphenyl)]*-5-chloro-1*H*-indole-2-carboxamide (89):



89

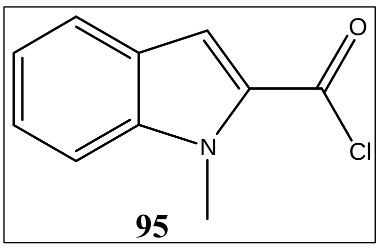
5-Chloroindole-2-carbonyl chloride (**87**, 0.40 g, 1.9 mmol) was added to a solution of 2-Amino-4'-methylbenzophenone (**13,** 0.43 g, 2.0 mmol) in 20 ml acetonitrile and 20 ml dry toluene. The mixture was refluxed for 12 hrs at 90 °C, then cooled. The organic layer was removed by evaporation under reduced pressure and the residue was crystallized using chloroform / methanol to afford a yellow solid (**89**, 0.34 g, 50 %); R_f: 0.48 (cyclohexane : ethylacetate, 7:3); m.p.: 260 °C; ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 11.94 (br s, 1H, NH-indole), 10.88 (br s, 1H, NHCO), 7.85 (d, *J* = 7.92 Hz, 1H, Ar-H), 7.75 (s, 1H, Ar-H), 7.71 – 7.57 (m, 3H, Ar-H), 7.50 – 7.36 (m, 2H, Ar-H), 7.35 – 7.24 (m, 3H, Ar-H), 7.23-7.13 (m, 2H, Ar-H), 2.35 (s, 3H, CH_3); ^{13}C-NMR (DMSO-d_6): δ = 196.03 (CO-ketone), 159.89 (CONH), 143.39, 138.33, 137.26, 135.11, 132.47 (CH-Ar), 131.41, 130.90 (CH-Ar), 130.47 (CH-Ar), 129.23 (CH-Ar), 127.38, 124.59 (CH-Ar), 124.47 (CH-Ar), 124.18 (CH-Ar), 121.30 (CH-Ar), 114.42 (CH-Ar), 103.66 (CH-Ar), 21.60 (CH-Ar) ppm; Anal. Calcd for $C_{23}H_{17}ClN_2O_2$: C, 71.04; H, 4.41; N, 7.20. Found: C, 70.88; H, 4.15; N, 7.10.

Synthesis of N-(9,10-dihydro-9,10-dioxoanthracen-1-yl)-5-chloro-1*H* indole- 2-carboxamide (90):



A mixture of 1-aminoanthraquinone (**17**, 0.40 g, 1.8 mmol) and 5-chloroindole-2-carbonyl chloride (**87**, 0.40 g, 1.9 mmol) was refluxed at 120 °C for 18 hrs in air condenser, then 1,4-dioxane (15 mL) was added. The reaction mixture was stirred for additional 24 hrs at RT, then cooled; the separated product was filtered, washed with ethanol, dried and crystallized from chloroform to give a dark red solid (**90**, 0.26 g, 33 %); R_f: 0.40 (cyclohexane : ethylacetate, 7:3); m.p.: □ 350 °C (decomposed); ^{1}H-NMR (300 MHz, DMSO-d_6): rotamers δ = 13.18 (br s, 1H, NH-indole), 12.25 (s, 1H, NHCO), 9.15 (d, *J*= 7.05, 1H, Ar-H), 8.38-7.25 (5m, 10H, Ar-H): [(8.33, d, *J*= 6.21 Hz; 8.21, d, *J*= 5.84 Hz; 8.35-7.80, m; 7.51, d, *J*= 8.7 Hz; 7.35, s, 7.29, d, *J*= 8.98 Hz)]; ^{13}C-NMR (DMSO-d_6): δ = 187.61 (CO-ketone), 182.60 (CO-ketone), 160.14 (CONH), 141.75, 136.48 (CH-Ar), 136.29, 135.31 (CH-Ar), 134.38, 134.18, 133.90, 133.07, 132.80, 128.50, 127.68 (CH-Ar), 127.03 (CH-Ar), 125.71, 125.37, 125.18 (CH-Ar), 122.51 (CH-Ar), 121.67 (CH-Ar), 118.31, 114.72 (CH-Ar), 103.65 (CH-Ar) ppm; Anal. Calcd for $C_{23}H_{13}ClN_2O_3$: C, 68.92; H, 3.27; N, 6.99. Found: C, 68.71; H, 3.25; N, 6.62.

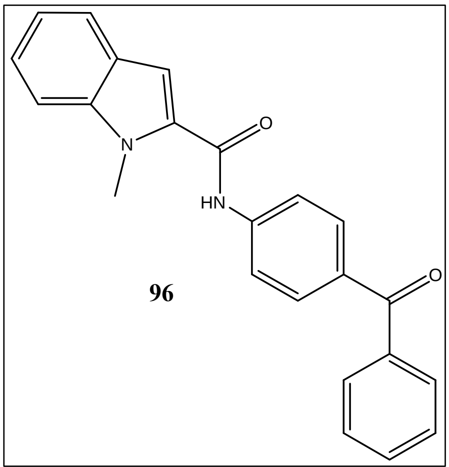
Synthesis of N-methyl-Indole-2-carbonyl chloride (96) (La Regina *et al.*, 2008):



A mixture of N-methyl indole-2-carboxylic acid (**95**, 1 g, 5.7 mmol) and $SOCl_2$ (3.6 mL, 50 mmol) in 25 mL of dry chloroform and drops of DMF was added in a round bottomed flask . The reaction mixture was refluxed for 24 hrs at 80 °C till the completion of the

reaction. Solvent was evaporated under vacuum, then chloroform was added twice and evaporated again to dryness in order to remove the excess of thionyl chloride present, to afford acid chloride (**95**, 0.95 g , 86%) as a light purple precipitate.

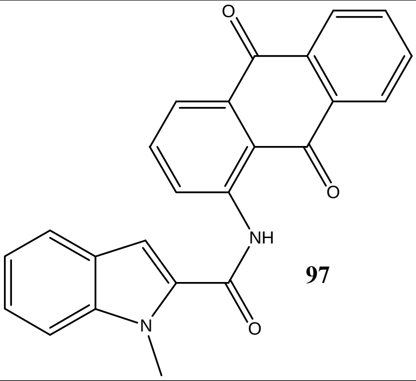
Synthesis of N-[4-benzoylphenyl]-1-methyl-1*H*-indole-2-carboxamide (96):



96

N-methyl Indole-2-carbonyl chloride (**95**, 0.41 g, 2.1 mmol) was added to a solution of 4-aminobenzophenone (**5**, 0.42 g, 2.1 mmol) and $NaOC_2H_5$ (0.35 g, 5.1 mmol) in 30 ml dry $CHCl_3$. The mixture was refluxed for 24 hrs at 100 °C, then cooled, washed with 1N HCl, followed by 1M sodium hydroxide and brine. The organic layer was taken, dried over $MgSO_4$ anhydrous and filtered. The organic layer was removed by evaporation under reduced pressure and the residue was purified by column chromatography using chloroform / methanol (95:5) as eluent to afford a dark brown mixture (**96**, 60 mg, 8%); R_f: 0.72 (cyclohexane : ethylacetate, 6:4); m.p.: 245 °C (decomposed); ^{1}H-NMR (300 MHz, DMSO-d_6) rotamers: δ = 11.12 + 10.05 (2br s, 1H, NHCO), 8.20 – 6.80 (m, 14H, Ar-H), 4.40 (s, 3H, N-CH_3); ^{13}C-NMR (DMSO-d_6): δ = 195.11 (CO-ketone), 163.25 (CONH), 138.77, 137.87, 132.85 (CH-Ar), 131.68 (CH-Ar), 131.09, 129.90 (CH-Ar), 128.99 (CH-Ar), 128.57, 127.65, 122.03, 121.78, 121.09, 120.37, 119.57 (CH-Ar), 111.76, 32.08 (CH_3) ppm; Anal. Calcd for $C_{23}H_{18}N_2O_2$: C, 77.95; H, 5.12; N, 7.90. Found: C, 78.11; H, 5.40; N, 7.98.

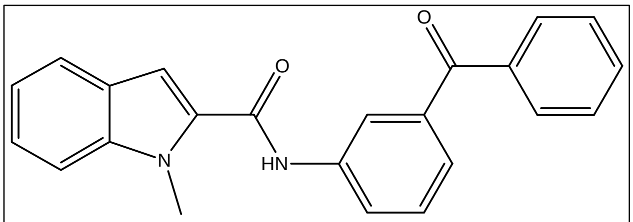
Synthesis of N-(9,10-dihydro-9,10-dioxoanthracen-1-yl)-1-methyl-1*H* indole- 2-carboxamide (97):



97

A mixture of 1-aminoanthraquinone (**17**, 0.40 g, 1.7 mmol) and N-methyl indole-2-carbonyl chloride (**95**, 0.40 g, 2.1 mmol) was refluxed at 120 °C for 18 hrs in air condenser, then 1,4-dioxane (15 mL) was added. The reaction mixture was stirred for additional 24 hrs at RT, then cool; the separated product was filtered, washed with ethanol, dried and crystallized from chloroform to afford a brown solid (**97**, 50 mg, 8%); R_f: 0.89 ($CHCl_3$: MeOH, 9:1); m.p: □ 300 °C (decomposed); ^{1}H-NMR (300 MHz, DMSO-d_6) rotamers: δ = 13.12, 12.98 (2br s, 1H, NHCO), 9.11 (s, 1H, Ar-H), 8.27 – 7.04 (m, 11H, Ar-H), 4.10 (s, 3H, N-CH_3); Anal. Calcd for $C_{24}H_{16}N_2O_3$: C, 75.78; H, 4.24; N, 7.36. Found: C, 75.55; H, 4.47; N, 7.68.

Synthesis of N-[3-benzoylphenyl]-1-methyl-1*H*-indole-2-carboxamide (98):



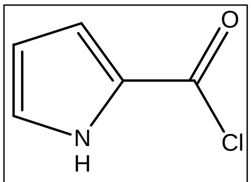
98

A mixture of N-methyl indole-2-carbonyl chloride (**95**, 0.41 g, 2.12 mmol) in 15 ml acetonitrile was added to a solution of 3-aminobenzophenone (**9**, 0.40 g, 2.03 mmol) in 20 ml dry toluene. The mixture was refluxed for 12 hrs at 100 °C, then cooled. The organic layer was removed by evaporation under reduced pressure and the residue was crystallized using chloroform / methanol (10ml, 9:1), to afford a black solid (**98**, 90 mg, 13%); R_f: 0.58 (cyclohexane : ethylacetate, 7:3); m.p.: 250 °C (decomposed); ^{1}H-NMR (300 MHz, DMSO-d_6) rotamers: δ = 10.95 + 10.52 (br s, 1H, NHCO), 8.22 – 8.00 (m, 2H, Ar-H), 7.90 – 6.85

(m, 12H, Ar-H), 3.92 (s, 3H, N-CH_3); [13]C-NMR (DMSO-d_6) rotamers: δ = 198.32 (CO-ketone), 163.37 (CONH), 141.94, 141.52,140.19, 139.77, 135.37 (CH-Ar), 134.35, 132.30 (CH-Ar), 132.06 (CH-Ar), 131.66 (CH-Ar), 131.25 (CH-Ar), 128.13, 127.40 (CH-Ar), 126.79 (CH-Ar), 126.67 (CH-Ar), 124.47 (CH-Ar), 123.72 (CH-Ar), 123.02 (CH-Ar), 113.28 (CH-Ar), 108.68 (CH-Ar), 34.15 (CH_3) ppm; Anal. Calcd for $C_{23}H_{18}N_2O_2$: C, 77.95; H, 5.12; N, 7.90. Found: C, 77.77; H, 4.93; N, 7.76.

6.11. Removal of benzene group from indole carboxamides group (Series H):

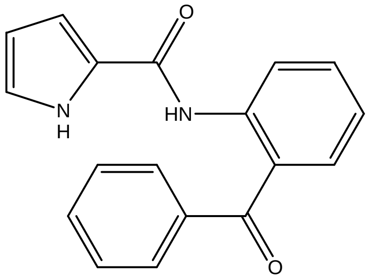
Synthesis of pyrrole-2-carbonyl chloride (100) (Boatman and Whitlock, 1976):



100

A mixture of pyrrole-2-carboxylic acid (**99**, 1 g, 9.01 mmol) and $SOCl_2$ (3.6 mL, 50 mmol) in 25 mL of dry DCM was added in a round bottomed flask. The reaction mixture was refluxed for 24 hrs at 50 °C till the completion of the reaction. Solvent was evaporated under vacuum, then dry toluene was added twice and evaporated again to dryness in order to remove the excess of thionyl chloride present, to afford acid chloride (**100**, 0.95 g , 81%) as a dark green solid; R_f: 0.42 ($CHCl_3$/ MeOH/ acetic acid, 94:5:1); m.p.: 90 °C (decomposed); [1]H-NMR (300 MHz, DMSO-d_6): δ = 11.65 (br s, 1H, NH-pyrrole), 6.94 (d, *J* = 3.0 Hz, 1H, H-5), 6.72 (m, 1H, H-3), 6.13 (d, *J* = 3.0 Hz, 1H, H-4); [13]C-NMR (DMSO-d_6): δ = 162.29 (CO-Cl), 123.80 (C-2), 123.37 (C-5), 115.11 (C-3), 109.71 (C-4) ppm; Anal. Calcd for C_5H_4ClNO: C, 46.36; H, 3.11; N, 10.81. Found: C, 46.87; H, 3.09; N, 11.08.

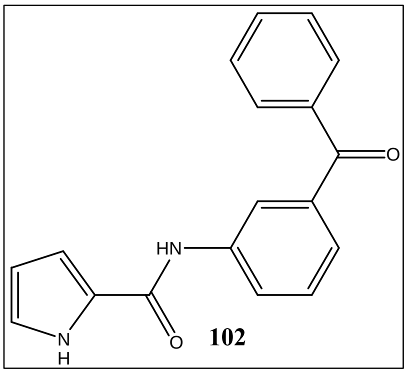
Synthesis of N-[2-benzoylphenyl]-1*H*-pyrrole-2-carboxamide (101):



101

A mixture of pyrrole-2-carbonyl chloride (**100**, 0.42 g, 3.2 mmol) in 15 ml acetonitrile was added to a solution of 2-aminobenzophenone (**11**, 0.35 g, 1.8 mmol) in 20 ml dry 1,4 dioxane. The mixture was refluxed for 12 hrs at 120 °C, then cooled. The organic layer was removed by evaporation under reduced pressure and the residue was crystallized using chloroform / methanol to afford a black solid (**101**, 0.20 g, 38%); R_f: 0.63 (cyclohexane: ethylacetate, 6:4); m.p.: 182 °C; ^{1}H-NMR (300 MHz, DMSO-d_6): δ= 11.70 (br s, 1H, NH-pyrrole), 10.56 (br s, 1H, NHCO), 7.91 (d, *J* = 7.39 Hz, 1H, Ar-H), 7.74 (d, *J* = 6.20 Hz, 2H, Ar-H), 7.68 – 7.55 (m, 2H, Ar-H), 7.51 (d, *J* = 6.57 Hz, 2H, Ar-H), 7.43 (d, *J* = 6.98 Hz, 1H, Ar-H), 7.25 (dd, *J* = 6.40, 6.40 Hz, 1H, Ar-H), 6.92 (s, 1H, Ar-H), 6.88 (s, 1H, Ar-H), 6.14 (s, 1H, Ar-H); ^{13}C-NMR (DMSO-d_6): δ = 196.62 (CO-ketone), 159.23 (CONH), 138.08, 137.96, 132.87 (CH-Ar), 132.84 (CH-Ar), 131.34 (CH-Ar), 130.19 (CH-Ar), 129.31, 128.64 (CH-Ar), 126.08, 123.66 (CH-Ar), 123.37 (CH-Ar), 111.65 (CH-Ar), 109.54 (CH-Ar) ppm; Anal. Calcd for $C_{18}H_{14}N_2O_2$: C, 74.47; H, 4.86; N, 9.65. Found: C, 74.20; H, 4.55; N, 9.42.

Synthesis of N-[3-benzoylphenyl]-1*H*-pyrrole-2-carboxamide (102):

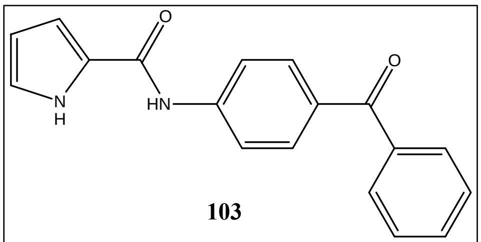


102

A mixture of pyrrole-2-carbonyl chloride (**100**, 0.42 g, 3.2 mmol) in 15 ml acetonitrile was added to a solution of 3-aminobenzophenone (**9**, 0.40 g, 2.0 mmol) in 20 ml dry toluene. The mixture was refluxed for 12 hrs at 90 °C, then cooled. The organic layer was removed by evaporation under reduced pressure and the residue was crystallized using chloroform / methanol to afford a black solid (**102**, 0.15 g, 25%);

R_f: 0.57 (cyclohexane: ethylacetate, 7:3); m.p.: 190 °C (decomposed); ^{1}H-NMR (300 MHz, DMSO-d_6) rotamers: δ = 11.98, 11.79 (2br s, 1H, NH-pyrrole), 10.40, 9.98 (2br s, 1H, NHCO), 8.01-6.15 (3m, 12H, Ar-H): [(8.15-8.05, m; 7.31-6.81, m; 6.32-6.10, m)]; ^{13}C-NMR (DMSO-d_6) rotamers: δ = 191.25 (CO-ketone), 164.30 (CONH), 133.13, 132.87, 131.50, 130.10, 129.90, 129.56, 129.04, 128.91, 125.19, 124.55, 124.15, 121.27, 119.63, 117.20, 113.52 ppm; HRMS (ESI, negative mode): m/z (M^+ - H^+) 289.09825 ($C_{18}H_{13}N_2O_2$) 289.09770; Anal. Calcd for $C_{18}H_{14}N_2O_2$: C, 74.47; H, 4.86; N, 9.65. Found: C, 74.19; H, 4.69; N, 9.71.

Synthesis of N-[4-benzoylphenyl]-1*H*-pyrrole-2-carboxamide (103):

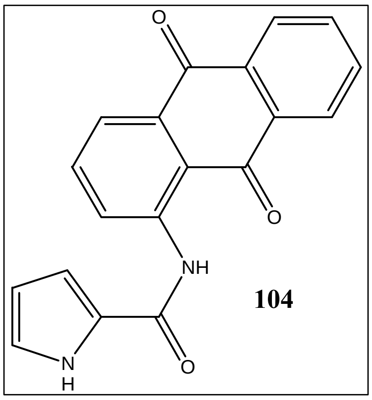


103

A mixture of pyrrole-2-carbonyl chloride (**100**, 0.40 g, 3.09 mmol) in 15 ml acetonitrile was added to a solution of 4-aminobenzophenone (**5**, 0.40 g, 2.03 mmol) in 20 ml dry toluene. The mixture was refluxed for 18 hrs at 90 °C, then cooled. The organic layer was removed by evaporation under reduced pressure and the residue was crystallized using chloroform / methanol to afford a black solid (**103**, 0.12 g, 20%); R_f: 0.60 ($CHCl_3$: MeOH: acetic acid, 90:10:1); m.p.: 185 °C (decomposed); ^{1}H-NMR (300 MHz, DMSO-d_6) rotamers: δ = 11.70, 11.82 (2br s, 1H, NH-pyrrole), 10.23, 9.85 (2br s, 1H, NHCO), 8.01-6.15 (5m, 12H, Ar-H): [(8.01-7.89, m; 7.85-7.45, m; 7.40-6.88, m; 6.80-6.45, m; 6.35-6.15, m)]; ^{13}C-NMR (DMSO-d_6) rotamers: δ = 195.15 (CO-ketone), 160.15, 159.95 (CONH), 154.21, 138.13, 133.04 (CH-Ar), 132.74 (CH-Ar), 131.50 (CH-Ar), 129.81 (CH-Ar), 129.22 (CH-Ar), 128.95 (CH-Ar), 128.65 (CH-Ar), 120.33, 119.34, 113.02 (CH-Ar) ppm; IR (thin film): ν = 3364 (NH-pyrrole), 3291 (NHCO), 3129, 1701 (CO), 1639 (CO), 1589, 1520, 1412, 1319, 1281, 1246, 1126, 845, 741 cm^{-1}; HRMS

(ESI, negative mode): m/z (M^+ - H^+) 289.09769 ($C_{18}H_{13}N_2O_2$) 289.09770; Anal. Calcd for $C_{18}H_{14}N_2O_2$: C, 74.47; H, 4.86; N, 9.65. Found: C, 74.28; H, 4.72; N, 9.43.

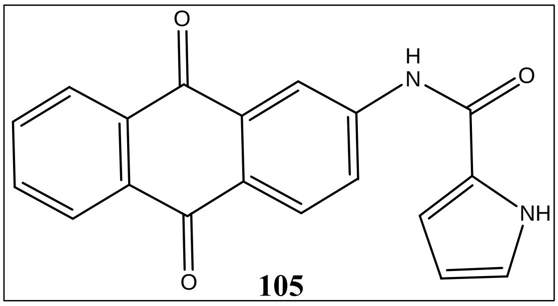
Synthesis of N-(9,10-dihydro-9,10-dioxoanthracen-1-yl)-1*H* pyrrole - 2-carboxamide (104) :



104

A mixture of 2-aminoanthraquinone (**17**, 0.42 g, 1.8 mmol) and pyrrole-2-carbonyl chloride (**100**, 0.42 g, 3.2 mmol) was refluxed at 90 °C for 18 hrs in air condenser, then 1,4-dioxane (15 mL) was added. The reaction mixture was stirred for additional 24 hrs at RT; the separated product was filtered, washed with ethanol, dried and crystallized from chloroform to give a dark green solid (**104**, 0.12 g, 21%); R_f: 0.42(cyclohexane: ethylacetate, 7:3); m.p.: □ 350 °C (decomposed); ^{1}H-NMR (300 MHz, DMSO-d_6): rotamors δ = 12.89 (br s, 1H, NH-pyrrole), 12.04 (br s, 1H, NHCO), 9.19-6.18 (6m, 10H, Ar-H): [(9.12, br s; 8.52-8.03, m; 7.92, br s; 7.57-7.17, m; 7.18-6.88, m; 6.32, br s)]; ^{13}C-NMR (DMSO-d_6): δ = 187.45 (CO-ketone), 182.68 (CO-ketone), 159.70 (CONH), 142.61, 136.35, 135.21, 134.30, 134.21, 133.89, 132.77, 128.91, 127.09, 126.38, 125.39, 124.87, 121.78, 117.65, 111.83, 110.29 ppm; IR (thin film): ν = 3291 (NH-pyrrole), 3209 (NHCO), 2924, 1667 (br CO), 1639 (CO), 1578, 1528, 1412, 1265, 1161, 1130, 1007, 845, 748 cm^{-1}; HRMS (ESI, positive mode): m/z (M^+ + Na^+) 339.07401 ($C_{19}H_{12}N_2NaO_3$) requires 339.07456.

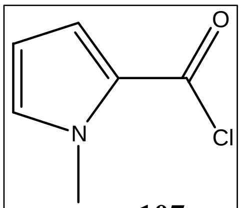
Synthesis of N-(9,10-dihydro-9,10-dioxoanthracen-2-yl)-1*H* pyrrole - 2-carboxamide (105) :



105

A mixture of 2-aminoanthraquinone (**19**, 0.45 g, 1.9 mmol) and pyrrole-2-carbonyl chloride (**100**, 0.45 g, 3.5 mmol) was refluxed at 90 °C for 18 hrs in air condenser, then 1,4-dioxane (15 mL) was added. The reaction mixture was stirred for additional 24 hrs at RT; the separated product was filtered, washed with ethanol, dried and crystallized from chloroform to give a black solid (**105**, 0.30 g, 47%); R_f: 0.65 ($CHCl_3$: MeOH: acetic acid, 90:10:1); m.p.: $>$ 350 °C (decomposed); ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 11.87 (br s, 1H, NH-pyrrole), 10.38 (br s, 1H, NHCO), 8.68-8.54 (m, 1H, Ar-H), 8.39-8.28 (m, 1H, Ar-H), 8.26-8.12 (m, 3H, Ar-H), 7.97-7.82 (m, 2H, Ar-H), 7.22 (br s, 1H, Ar-H), 7.06 (br s, 1H, Ar-H), 6.22 (s, 1H, Ar-H); ^{13}C-NMR (DMSO-d_6): δ = 183.08 (CO-ketone), 181.84 (CO-ketone), 159.89 (CONH), 145.69, 135.02 (CH-Ar), 134.65 (CH-Ar), 134.52, 133.67, 133.61, 128.86 (CH-Ar), 128.00, 127.21 (CH-Ar), 127.11 (CH-Ar), 125.89, 124.61 (CH-Ar), 124.21 (CH-Ar), 116.86 (CH-Ar), 113.04 (CH-Ar), 109.77 (CH-Ar) ppm; IR (thin film): ν = 3345 (NH-pyrrole), 3298 (NHCO), 1674 (br CO), 1647 (CO), 1524, 1423, 1288, 1231, 1119, 930, 845 cm^{-1}.

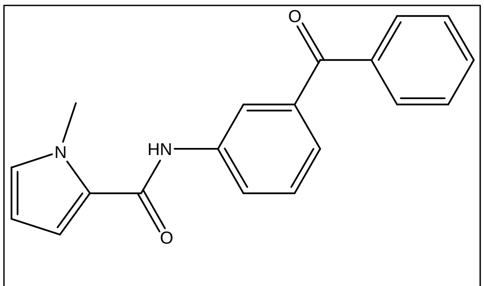
Synthesis of N-methyl-pyrrole-2-carbonyl chloride (107) (Bhattacharya and Thomas, 2001):



107

A mixture of N-methylpyrrole-2-carboxylic acid (**106**, 1 g, 7.99 mmol) and $SOCl_2$ (3.6 mL, 50 mmol) in 25 mL of dry DCM was added in a round bottomed flask. The reaction mixture was refluxed for 24 hrs at 50 °C till the completion of the reaction. Solvent was evaporated under vacuum, then dry toluene was added twice and evaporated again to dryness in order to remove the excess of thionyl chloride present, to afford acid chloride (**107**, 0.95 g , 83%) as a pale purple solid.

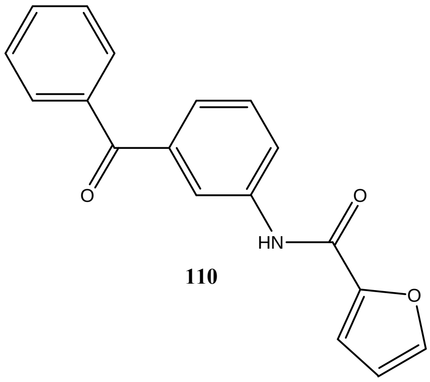
Synthesis of N-[3-benzoylphenyl]-1-methyl-1*H*-pyrrole-2-carboxamide (108):



108

A mixture of N-methyl-pyrrole-2-carbonyl chloride (**107**, 0.40 g, 2.8 mmol) in 15 ml acetonitrile was added to a solution of 3-aminobenzophenone (**9**, 0.40 g, 2.0 mmol) in 20 ml dry toluene. The mixture was refluxed for 12 hrs at 90 °C, then cooled. The organic layer was removed by evaporation under reduced pressure and the residue was crystallized using chloroform / methanol to afford a black solid (**108**, 80 mg, 13%); R_f: 0.38 (cyclohexane: ethylacetate, 7:3); m.p.: 195 °C (decomposed); ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 10.75 (br s, 1H, NHCO), 8.17 – 7.98 (m, 2H, Ar-H), 7.74 – 6.46 (m, 10H, Ar-H), 4.04 (s, 3H, N-CH_3); ^{13}C-NMR (DMSO-d_6): δ = 197 (CO-ketone), 161 (CONH), 143, 141, 140, 135 134, 133, 128, 127.40, 126, 124, 111, 106, 33 (CH_3) ppm.

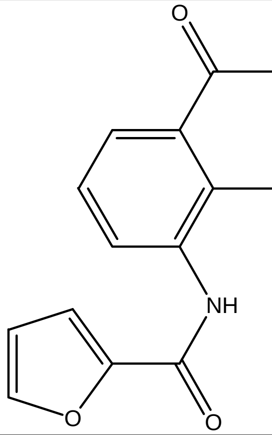
Synthesis of N-(3-benzoylphenyl)-2-furamide (110):



110

Furan-2-carbonyl chloride (**109**, 0.41 g, 3.1 mmol) was added to a solution of 3-aminobenzophenone (**9**, 0.35 g, 1.8 mmol) in 20 ml acetonitrile and 20 ml dry DCM. The mixture was refluxed for 12 hrs at 80 °C, then cooled. The organic layer was removed by evaporation under reduced pressure and the residue was crystallized using chloroform / methanol to afford a yellow solid (**110**, 0.13 g, 25 %); R_f: 0.70 ($CHCl_3$: MeOH, 9:1); m.p.: 181 °C; ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 10.52 (br s, 1H, NHCO), 8.21 (s, 1H, Ar-H), 8.12 (d, *J* = 8.1 Hz, 1H, Ar-H), 7.96 (s, 1H, Ar-H), 7.77 (d, *J* = 8.1 Hz, 2H, Ar-H), 7.74 – 7.67 (m, 1H, Ar-H), 7.64 – 7.50 (m, 3H, Ar-H), 7.48 – 7.40 (m, 2H, Ar-H), 6.71 (dd, *J* = 4.8, 1.5 Hz, 1H, Ar-H); ^{13}C-NMR (DMSO-d_6): δ = 196.05 (CO-ketone), 156.87 (CONH), 147.65, 146.45 (CH-Ar), 139.26, 137.82, 137.44, 133.19 (CH-Ar), 130.14 (CH-Ar), 129.46 (CH-Ar), 129.05 (CH-Ar), 125.31 (CH-Ar), 124.66 (CH-Ar), 121.76 (CH-Ar), 115.64 (CH-Ar), 112.69 (CH-Ar) ppm; Anal. Calcd for $C_{18}H_{13}NO_3$: C, 74.22; H, 4.50; N, 4.81. Found: C73.93; H, 4.34; N, 4.60.

Synthesis of N-(9,10-dihydro-9,10-dioxoanthracen-1-yl)-2-furamide (111):



111

A mixture of 1-aminoanthraquinone (**17**, 0.40 g, 1.8 mmol) and furan-2-carbonyl chloride (**109**, 0.45 g, 3.5 mmol) was refluxed at 120 °C for 18 hrs in air condenser, then 1,4-dioxane (15 mL) was added. The reaction mixture was stirred for additional 24 hrs at RT. The precipitate was filtered, washed with water, dried and crystallized from chloroform to give a dark red solid (**111**, 0.18 g, 32 %); R_f: 0.67 ($CHCl_3$: MeOH, 9:1); m.p.: 280 °C (decomposed); ^{1}H-NMR (300 MHz, DMSO-d_6) rotamers: δ = 13.18 (br s, 1H, NHCO), 9.15 (br s, 1H, Ar-H, H_3-furan), 8.35-8.10 (m, 2H, Ar-H), 7.94-7.72 (m, 4H, Ar-H), 7.46-7.12 (m, 2H, Ar-H), 6.95-6.61 (m, 1H, Ar-H); ^{13}C-NMR (DMSO-d_6) rotamers: δ = 192.82 (CO-ketone), 186.44 (CO-ketone), 161.22 (CONH), 148.09, 147.51, 147.22, 136.33, 135.25, 127.62, 126.96, 125.71, 122.59, 118.83, 118.18, 116.91, 113.52, 112.77, 112.56 ppm; Anal. Calcd for $C_{19}H_{11}NO_4$: C, 71.92; H, 3.49; N, 4.41. Found: C, 71.55; H, 3.63; N, 4.66.

7. Experimental model of hyperlipidemia:

7.1. Animals and treatments:

Two hundred and fifty adult male Wistar rats, weighing around 250 g, were bred in the animal house of the (faculty of pharmacy, Al-Zaytoonah University, Amman, Jordan), provided *ad libitum* access only to tap-water throughout the experimental duration 24 hrs. Rats were maintained in a 12 hrs light-dark cycle under conditions of constant humidity and temperature (22 ± 2 °C).

7.2. Triton model of hyperlipidemia:

Triton WR-1339 (Tyloxapol), a non-ionic detergent (oxyethylated tertiary octyl phenol formaldehyde polymer), has been widely used to induce acute hyperlipidaemia in animal models in order to investigate chemical or natural drugs. The accumulation of plasma lipid by Triton WR-1339 appears to be due to inhibition of lipoprotein and hepatic lipases activity. In addition to inhibition of LPL, Triton has a number of other physiologic effects related to lipoprotein. Triton has been shown to cause dissociation of apo A-I and apo C-II from HDL (Agren *et al.,* 2005). Therefore Triton WR-1339 (Tyloxapol, Sigma-Aldrich, USA) was dissolved in normal saline (PH 7.4) and administered intraperitoneally to the rats (≈250 mg/kg body weight) in order to induce hyperlipidaemia.

7.3. Experimental design:

Overnight fasted rats were randomly divided into five groups of six animals each. The first group, serving as a negative control group (CG) received an intraperitoneal administration of normal saline; the second group, serving as a positive control group (PCG) received an intraperitoneal administration of 4% DMSO; the third

hyperlipidaemic group (HG) received an intraperitoneal injection of Triton and gavaged with 4% DMSO (in distilled water). In the fourth group rats were intraperitoneally injected with Triton, followed by an intragastric administration of 57 μm of the target compounds dissolved in 4% DMSO. The last group (BF) was intraperitoneally injected with Triton and intragastrically treated with bezafibrate (100 mg/kg body weight) dissolved in 4% DMSO.

After 8 hrs of treatments, animals were anaesthetized with diethyl ether and blood was collected. The blood samples were immediately centrifuged (3000 rpm for 10 min) and the plasma was used for lipid analysis by an enzymatic method with an automatic analyzer (Model Erba XL-300, Germany, Mannheim, Germany).

7.4. Statistical analysis:

Results were expressed as mean ± SEM. Data obtained was analyzed using the Student's t-test and differences with $P<0.05$ were considered statistically significant.

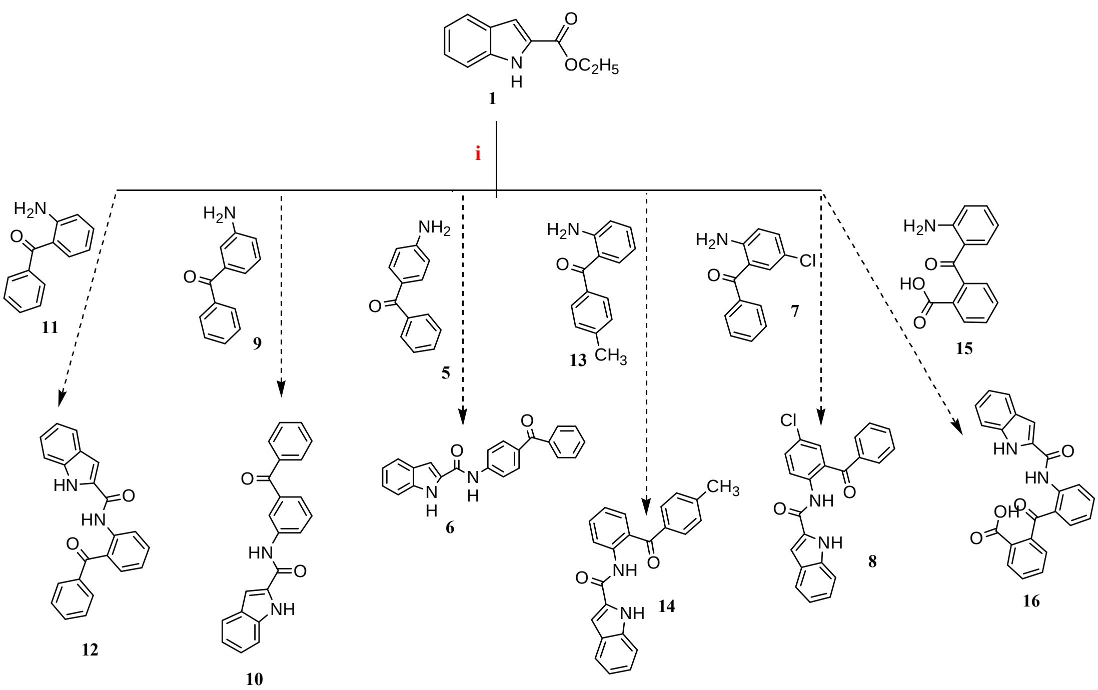
8. Results and Discussion:

8.1. Chemistry:

Unsubstituted indole-benzophenone 2-carboxamides group (Series A):

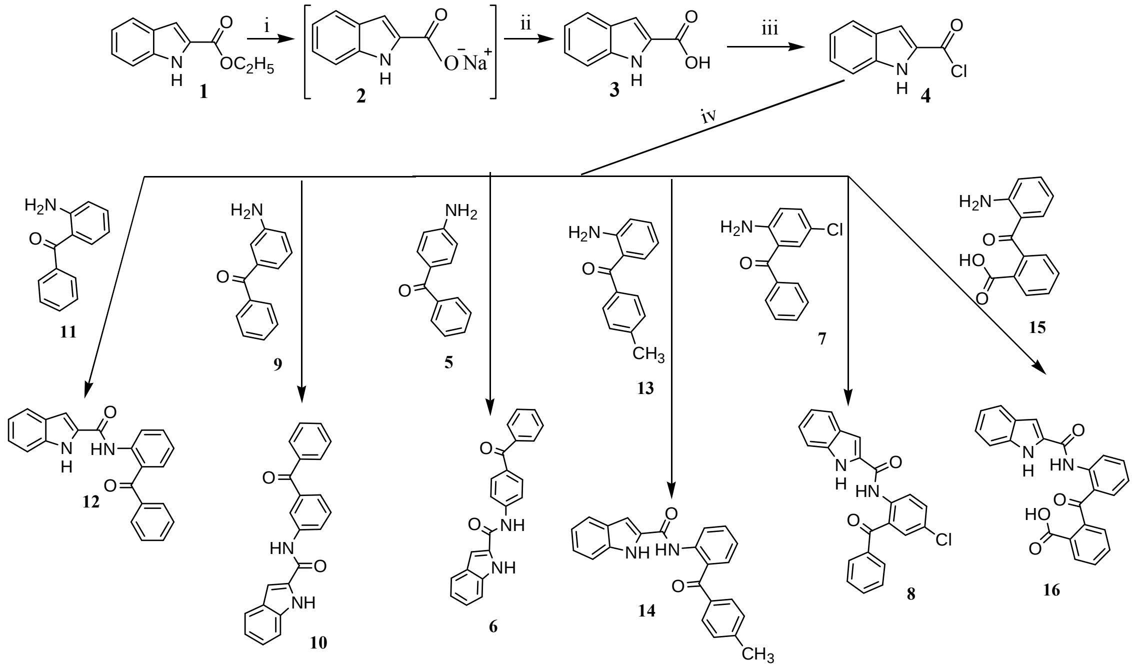
Benzophenone carboxamides derivatives (**6, 8, 10, 12, 14** and **16**) were prepared using two major pathways (Scheme 1).

The ester pathway involved coupling of ethyl-1*H*-indole-2-carboxylate (**1**) with the corresponding aminobenzophenones, using sodium ethoxide ($NaOC_2H_5$) as the base. Upon refluxing in DMF for 1-4 days, the corresponding amide (**6, 8, 10, 12, 14** and **16**) were formed but in low yield (16%). Different conditions were further used to improve the yield, such as using different {bases; $NaOC_2H_5$, TEA and pyridine; solvents; DMF, DMSO, $CHCL_3$, DCM; and temperatures} but all furnished the same results.



Scheme 1. Preparation of indole-benzophenone-2-carboxamides (**6, 8, 10, 12, 14, 16**). Starting from ethyl-indole-2-carboxylate (**1**). i: $NaOC_2H_5$, DMF, 100-150 °C.

An alternative procedure, involved indole-2-carbonyl chloride (**4**), was used to prepare the target compounds, with higher yields (Scheme 2). Ethyl-indole-2-carboxylate (**1**) was treated with sodium hydroxide in methanol, to get the sodium indole salt (**2**). The salt was then treated with 2N HCl in methanol to produce the indole-2-carboxylic acid (**3**), which was converted to the indole-2-carbonyl chloride (**4**). Next, indole-2-carbonyl chloride (**4**) was treated with aminobenzophenone derivatives in the presence of $NaOC_2H_5$ in DCM or $CHCl_3$ over 8-24 hrs at 80 °C, and the mixture was purified using column chromatography and recrystallized to afford the desired compounds (**6, 8, 10, 12, 14 and 16**) in higher yields (30-60%).



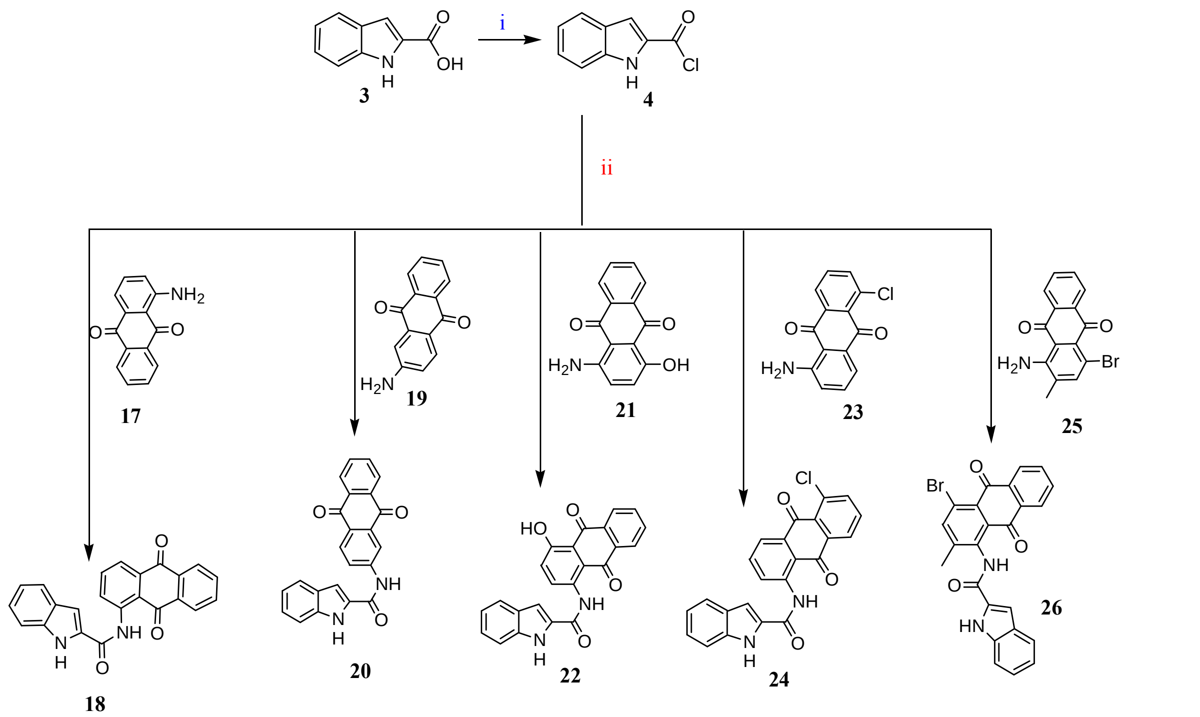
Scheme 2. Preparation of indole-benzophenone-2-carboxamides (**6, 8, 10, 12, 14, 16**) starting from indole-2-carbonyl chloride. i: NaOH, MeOH; ii: 2N HCl in MeOH; iii: $SOCl_2$, 70-80 °C, $CHCl_3$; iv: $NaOC_2H_5$, DCM or $CHCl_3$, 80 °C.

All these compounds were characterized by NMR, IR and MS and were consistent with expected positions/ peaks. ^{1}H-NMR spectra of the targets have shown two new broad singlet in all targets at ≈ 12 and 10, for NH-indole and HN-amide respectively. These two peaks confirmed that the amide linkage had occurred and confirmed the structure. ^{13}C-NMR spectra on the other hand, have shown the exact numbers of carbon peaks spread all over the aromatic region, with a distinct amide peak (CONH) at ≈ 160 ppm and another distinctive peak for the benzophenone carbonyl carbon (C=O) at around 200 ppm, again these two peaks confirmed the structures of the derivatives. Further proof was provided by MS, showing the exact molecular ion of each derivative.

All these compounds have been evaluated for their antihyperlipidemic activities and only compounds **6** and **10** showed activity (see biological part)

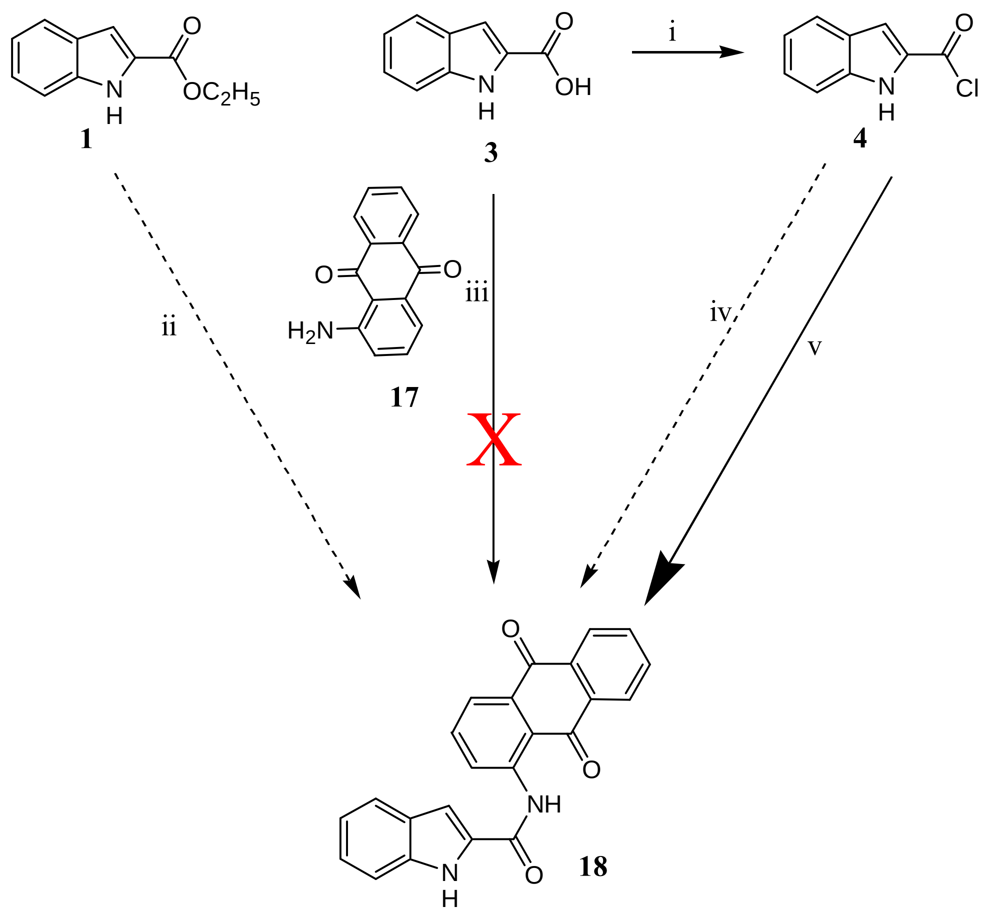
Unsubstituted indole-anthraquinone carboxamides group (Series B):

In this series four procedures have been performed in an attempt to produce the target compounds (**18, 20, 22, 24** and **26**, Scheme 3).



Scheme 3. Preparation of indole-anthraquinone-2-carboxamide derivatives (**20, 22, 23, 24** and **26**) starting from 5-indole-2-carbonyl chloride. i: $SOCl_2$, 70-80 °C, $CHCl_3$; ii: refluxed at 120 °C.

The first procedure was performed using the ester pathway (Scheme 4). Ethyl-1*H*-indole-2-carboxylate (**1**) was treated with 1-aminoanthraquinone (**17**) in the presence of $NaOC_2H_5$ in DMF at 150 °C. The reaction mixture was purified using column chromatography to afford the desired compounds, but unfortunately in trace amounts (5%). Other conditions were attempted with ester pathway, using DMSO, DMF as solvents with other bases such as pyridine and $NaOCH_3$, but all failed to give an isolable amount.



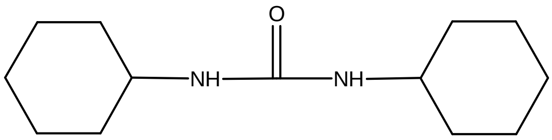
Scheme 4. Preparation of indole-anthraquinone-2-carboxamide (**18**). i: $SOCl_2$, 70-80 °C, $CHCl_3$; ii: $NaOC_2H_5$, DMF, 150 °C; iii: DCC, THF, RT; iv: TEA, DMF, RT; v: refluxed at 120 °C

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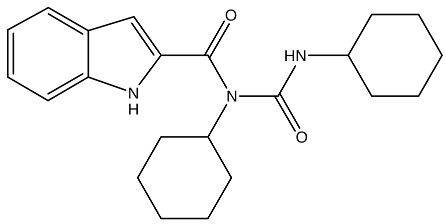
In the second procedure (Scheme 4), indole-2-carbonyl chloride (**4**) was added to (**17**) in the presence of TEA and DMF at RT. The reaction mixture was purified using column chromatography to afford the target compound but again in very low yield. The same procedure has been conducted at higher temperature □ 100 °C, or using other bases (e.g $NaOC_2H_5$) but did not provide significant improvement in the yield.

The third procedure (Scheme 4), involved coupling (**17**) with 1,3-dicyclohexylcarbodiimide (DCC) in THF, followed by stirring for 24 hrs at RT. The

procedure failed to produce the target compound. The DCC reaction was also carried out at different condition and higher temperature, yielding always two major side products (intermediates **A** and **B**) but not the targets (Figure 29). Both side products were isolated and characterized by NMR. The NMR of the major side product (dicyclohexane urea, **A**) is described in the experimental part.



A



B

Figure 29: The side products (intermediates **A** and **B**), of the DCC reaction with 1-aminoanthraquinone (**17**) to produce the corresponding amide.

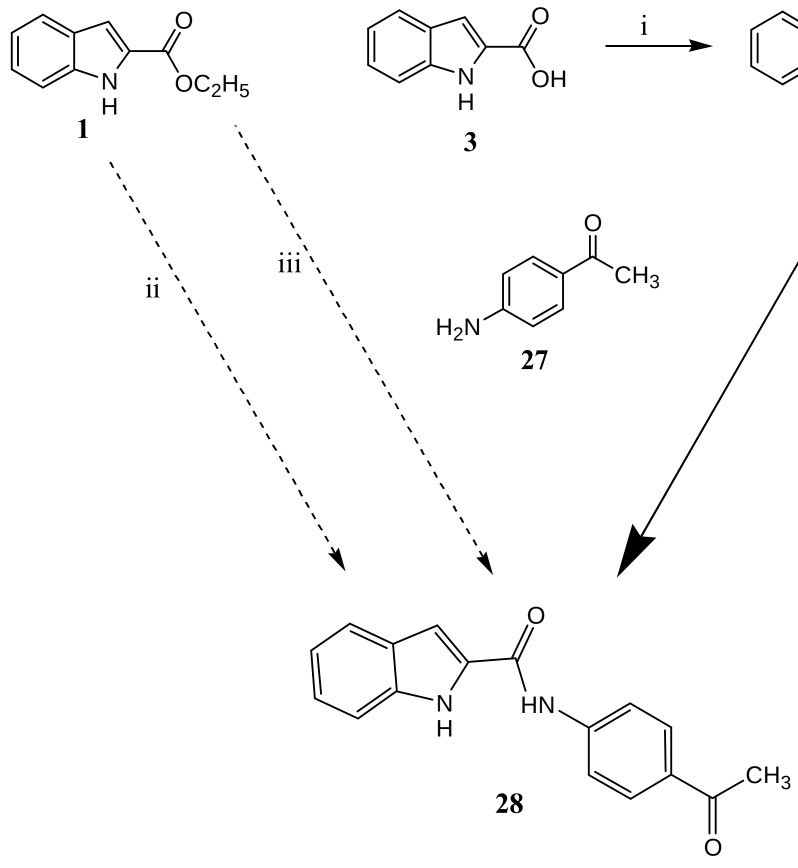
The last procedure (Scheme 3 and 4) which was adopted for the rest of the anthraquinone derivatives, was based on fusion at high temperature. Indole-2-carbonyl chloride (**4**) was thoroughly mixed with aminoanthraquinone derivatives, refluxed at 120 °C for 18 hrs in air condenser, then, 1,4-dioxane was added and stirred for additional 24 hrs at RT. The products were filtered and recrystalized from chloroform/methanol to give the desired compounds (**18, 20, 22, 24** and **26**) (Scheme 3 and 4).

All these compounds were characterized by NMR, IR and MS and were consistent with expected positions/ peaks. ^{1}H-NMR spectra showed the typical NH-indole and NH-

amide peaks at δ ≈13 and 11 ppm respectively, with a repetitive pattern of peaks for each proton, indicating rotameric phenomenon. On the other hand, the two carbonyl of the anthraquinone appeared as two singlet peaks at δ ≈ 180 and 190 ppm, where as the amide carbonyl was also indicative peak at around 160 ppm. Apart from compound **20**, anthraquinone carboxamide derivatives (compounds **18, 22, 24** and **26)** were found to exhibit antihyperlipidemic activity.

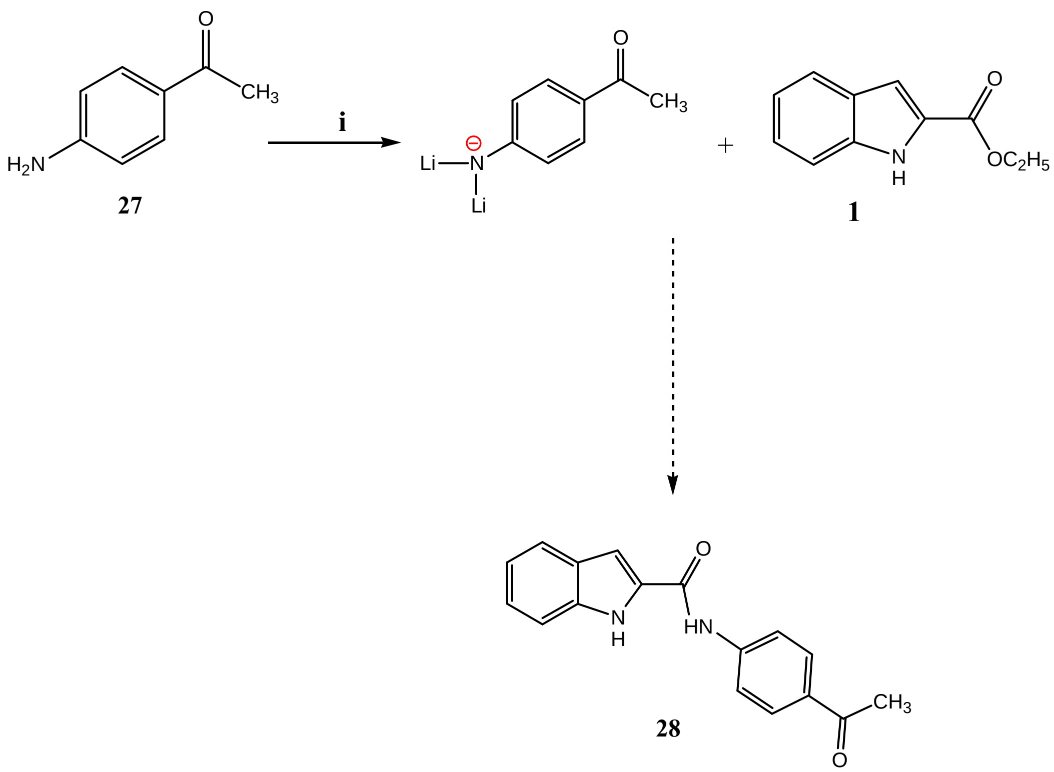
Unsubstituted indole-acetophenone carboxamides and derivatives (Series C):

Several attempts were made to synthesize the target acetophenone-2-carboxamide derivatives (**28, 30, 32** and **34**, Scheme 5).



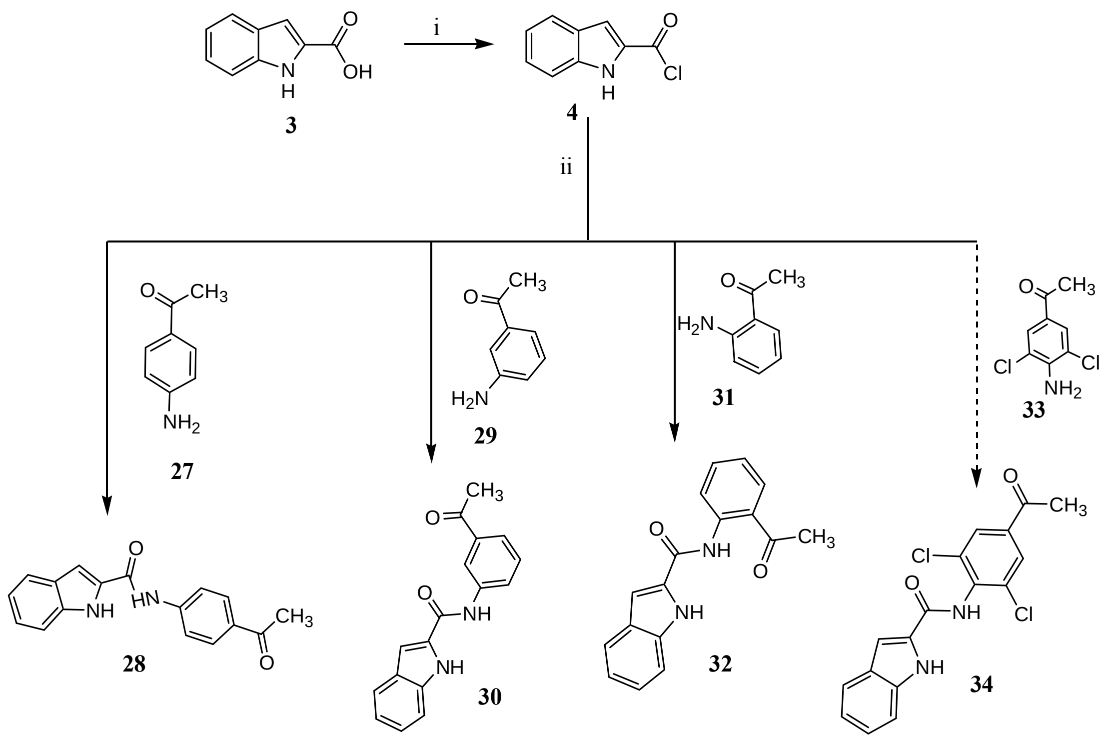
Scheme 5. Preparation of indole-4-amino-acetophenone-2-carboxamide (**28**). i: $SOCl_2$, 70-80 °C, $CHCl_3$; ii: $NaOC_2H_5$, DMF, 150 °C; iii: BuLi, THF, -78°C; iv: TEA, DCM, RT.

In an attempt to prepare indole-4-amino-acetophenone-2-carboxamide (**28**), 4-aminoacetophenone (**27**) was treated with ethyl-1*H*-indole-2-carboxylate (**1**), using sodium ethoxide in DMF at 150 °C. The reaction in this case failed to give reasonable yield and took longer time (Scheme 6). Alternatively, the search for stronger base was sought. The strong base BuLi was used to generate a strong nucleophilic amine by mixing with 4-aminoacetophenone (**27**) in dry THF. After cooling to -78 °C, ethyl-1*H*-indole-2-carboxylate (**1**) was added dropwise, and stirred for 1 hour at the same temperature. The reaction mixture was worked up and purified using column chromatography to afford the target compound but again in very low yield (Scheme 6). Since this procedure requires anhydrous condition and to keep the temperature at -78°C all the time, it facilitated parallel side reactions, giving rise many side products.



Scheme 6. Preparation of indole-4-amino-acetophenone-2-carboxamide (**28**), bu lithiation. i: excess BuLi, THF, -78°C

The final procedure involved treating indole-2-carbonyl chloride (**4**), with amino-acetophenone derivatives in the presence of TEA in DCM at RT. Then, the reaction mixture was purified using column chromatography to afford the target compounds (**28, 30, 32** and **34**) in a good yield (25-39%) (Scheme 7).



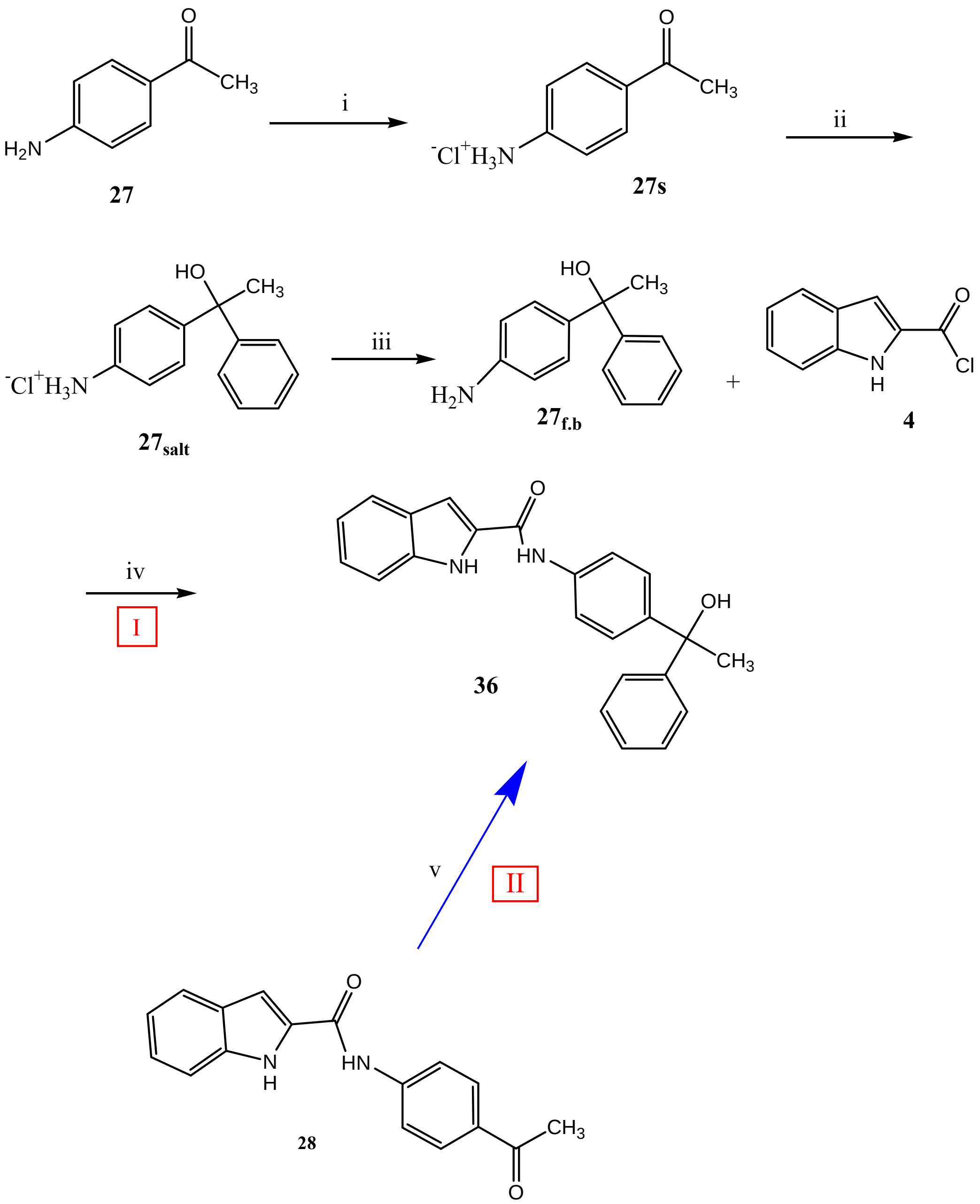
Scheme 7. Preparation of indole-acetophenone-2-carboxamide derivatives (**28, 30, 32** and **34**) starting from indole-2-carbonyl chloride. i: $SOCl_2$, 70-80 °C, $CHCl_3$; ii: Et_3N, DCM, RT.

All these compounds (**28, 30, 32** and **34**), were characterized by NMR, IR and MS and were consistent with expected positions/ peaks. However, non was found active as antihyperlipidemic agent. This prompted to modify the final product by adding more lipophilic group at carbonyl of the acetophenone moiety, by two routes: Grignard pathway (**A**) and Schiff base formation (**B**) (Scheme 8 and 9, respectively).

Grignard pathway (A): To prepare the target compound N-[4-(1-hydroxy-1-phenylethyl)phenyl]-1*H*-indole-2-carboxamide (**36**), two procedures were followed (Scheme 8; I and II), in which the second procedure (II), that started with the N-(4-acetylphenyl)-1*H*-indole-2-carboxamide (**28**) gave better yield.

The first procedure (I) involved protection of 4-aminoacetophenone (**27**) followed by adding phenylmagnesium chloride (**GR**) in dry ether, to produce the Grignard alcoholic derivative 1-(4-aminophenyl)-1-phenylethanol (**27_{sg}**). Next, the free alcoholic amine (**$27_{f.b}$**) was treated with indole-2-carbonyl chloride (**4**) in the presence of TEA in DCM at 80 °C, giving the target compound (**36**) in a very low yield (4%).

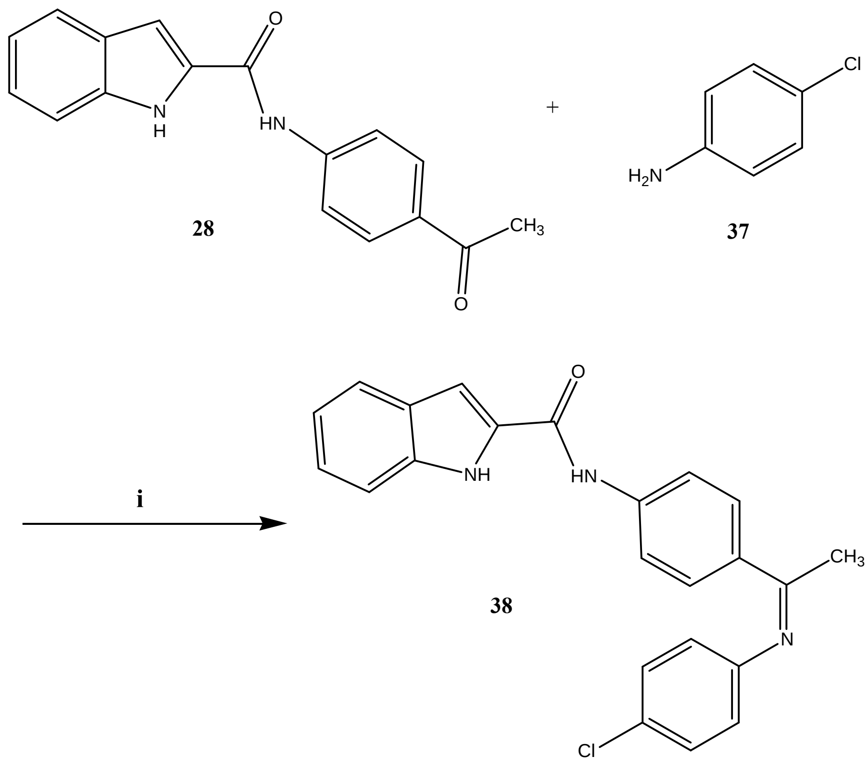
The second procedure (II), N-(4-acetylphenyl)-1*H*-indole-2-carboxamide (**28**), was stirred with phenylmagnesium chloride (**GR**) in dry ether to give the desired compound N-[4-(1-hydroxy-1-phenylethyl)phenyl]-1*H*-indole-2-carboxamide (**36**), in a reasonable yield (38%), which was active upon testing as anti-dyslipidemic agent.



Scheme 8. Preparation of N-[4-(1-hydroxy-1-phenylethyl)phenyl]-1*H*-indole-2-carboxamide (**36**). i: HCl, DCM, RT; ii: phenylmagnesium chloride **(GR)**, dry ether, RT; iii: NaOH, DCM; iv: TEA, DCM, 80 °C; v: GR, dry ether, RT.

Schiff base formation (B): Another optimization to obtain an active derivative, involved Schiff base formation on the carbonyl of the acetophenone moiety to produce N-{ 4-[-N-(4-chlorophenyl) ethanimidoyl] phenyl}-1*H*-indole-2-carboxamide (**38**) (Scheme 9). The preparation of compound (**38**), was carried out by simply refluxing N-(4-acetylphenyl)-1*H*-indole-2-carboxamide (**28**), with p-chloro-aniline (**37**) in absolute

methanol, at 75 °C, using drops of HCl as a catalyst. The precipitated product was filtered and recrystallized to afford the pure imine derivative (**38)** with a good yield, giving also an active hit as anti-dyslipidemic agent.

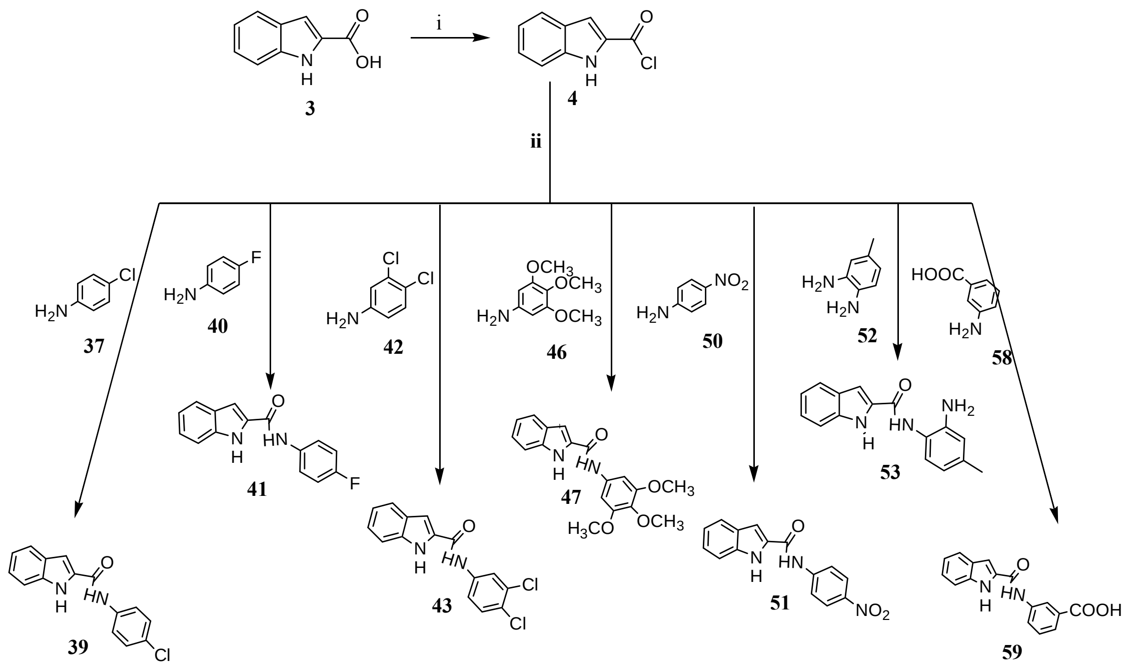


Scheme 9. Preparation of N-{ 4- [(1Z)–N-(4-chlorophenyl) ethanimidoyl] phenyl}-1*H*-indole-2-carboxamide (**38**) starting from N-(4-acetylphenyl)-1*H*-indole-2-carboxamide (**28**). i: methanol, drops HCl, 75 °C.

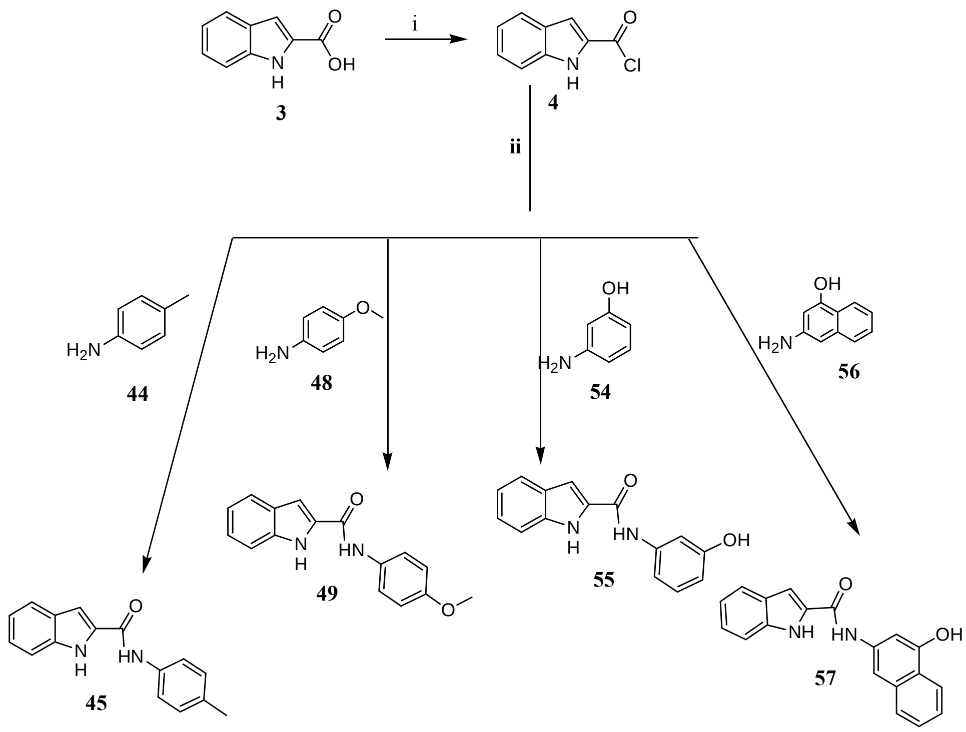
The last two derivatives (**36** and **38**), which involved additional phenyl group to acetophenone; in a similar way to the active benzophenone class, converted the compounds from inactive to active hit, which implicated the importance of the presence of this group for dyslipidemic activities.

Unsubstituted and substituted indole-benzene carboxamides group (Series D):

The preparation of the target compounds (**39, 41, 43, 47, 51, 53** and **59**) was simply performed by stirring indole-2-carbonyl chloride (**4**), with the corresponding aniline derivatives (**37, 40, 42, 46, 50, 52** and **58**) in DCM and TEA at RT (Scheme 10). Target compounds **(45, 49, 55** and **57)**, were synthesized by refluxing indole-2-carbonyl chloride (**4**), with the corresponding simple aromatic amines at 80 °C, in hot toluene and 1,4-dioxane (Scheme 11). Apart from compound (**59**), all target compounds were obtained in reasonable yield.

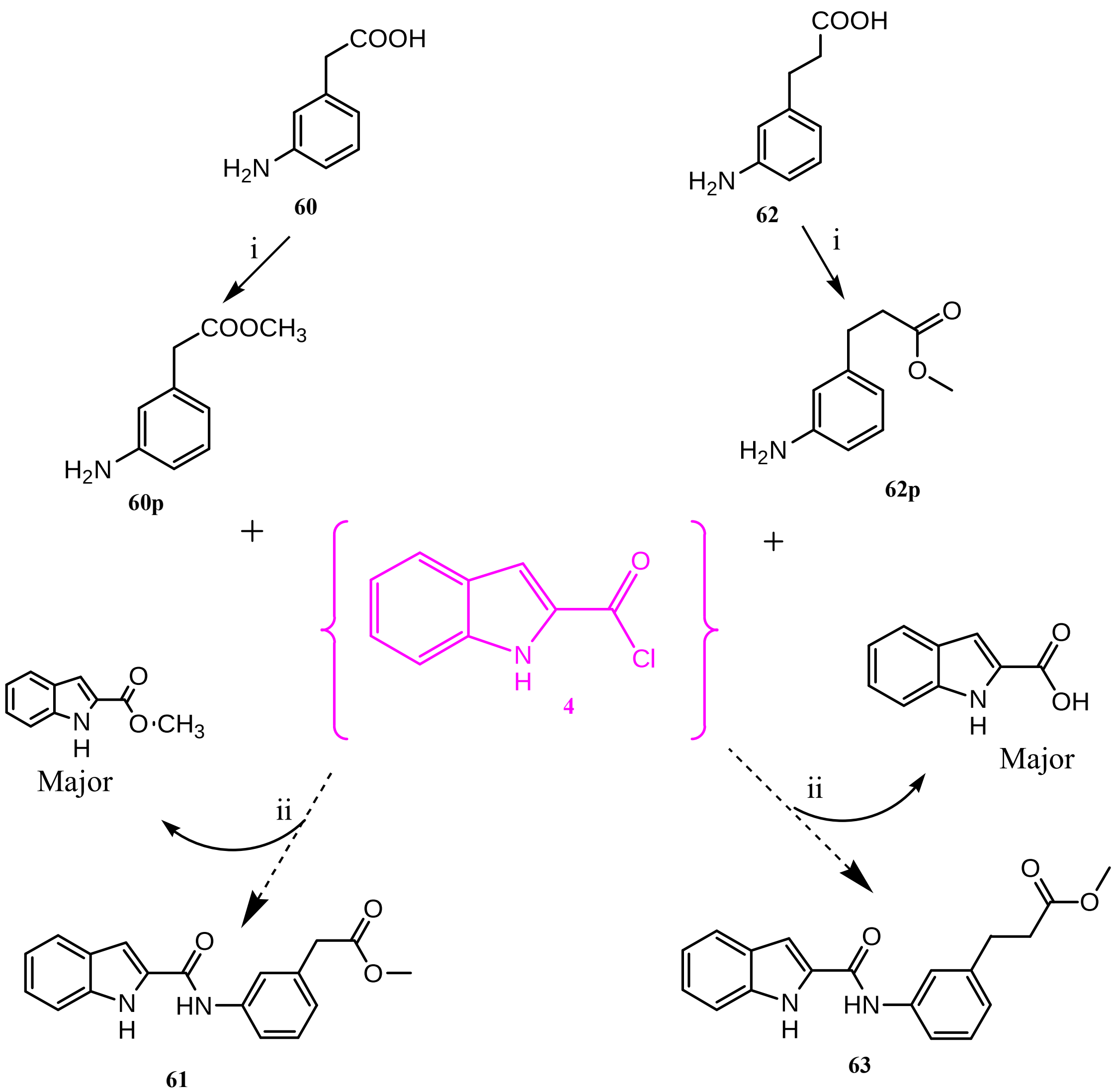


Scheme 10. Preparation of indole-aniline-2-carboxamide derivatives (**39, 41, 43, 47, 51, 53** and **59**). i: $SOCl_2$, 70-80 °C, $CHCl_3$; ii: TEA, DCM, RT.



Scheme 11. Preparation of indole-aniline-2-carboxamide derivatives (**45, 49, 55** and **57**). i: $SOCl_2$, 70-80 °C, $CHCl_3$; ii: dioxane, toluene, 80°C.

Compound methyl-{3-[(1*H*-indole-2-yl-carbonyl)-amino] phenylacetate (**61**) and compound methyl 3-{3-[(1*H*-indole-2-ylcarbonyl)-amino] phenylpropanoate (**63**), could not be prepared using previous procedures. Therefore, many procedures were attempted using different solvents, bases, reaction conditions, but the yield of these compounds resulted unsatisfactorily and not pure. An alternative method shown in (Scheme 12) was used. Both Meta-amino-phenyl acetic acid (**60**) and Meta-amino-phenyl propionic acid (**62),** were protected by converting to their methyl ester counterparts compounds (**60p** and **62p**) respectively, using thionyl chloride and methanol at 80 °C. Next, the methyl esters compounds (**60p** and **62p**), were treated with indole-2-carbonyl chloride (**4**) in the presence of TEA in DMF 120 °C. Then, the reaction mixtures were purified using column chromatography to afford the target compounds (**61** and **63**) in a very low yield, their peaks were picked up from the mixture as minor products.

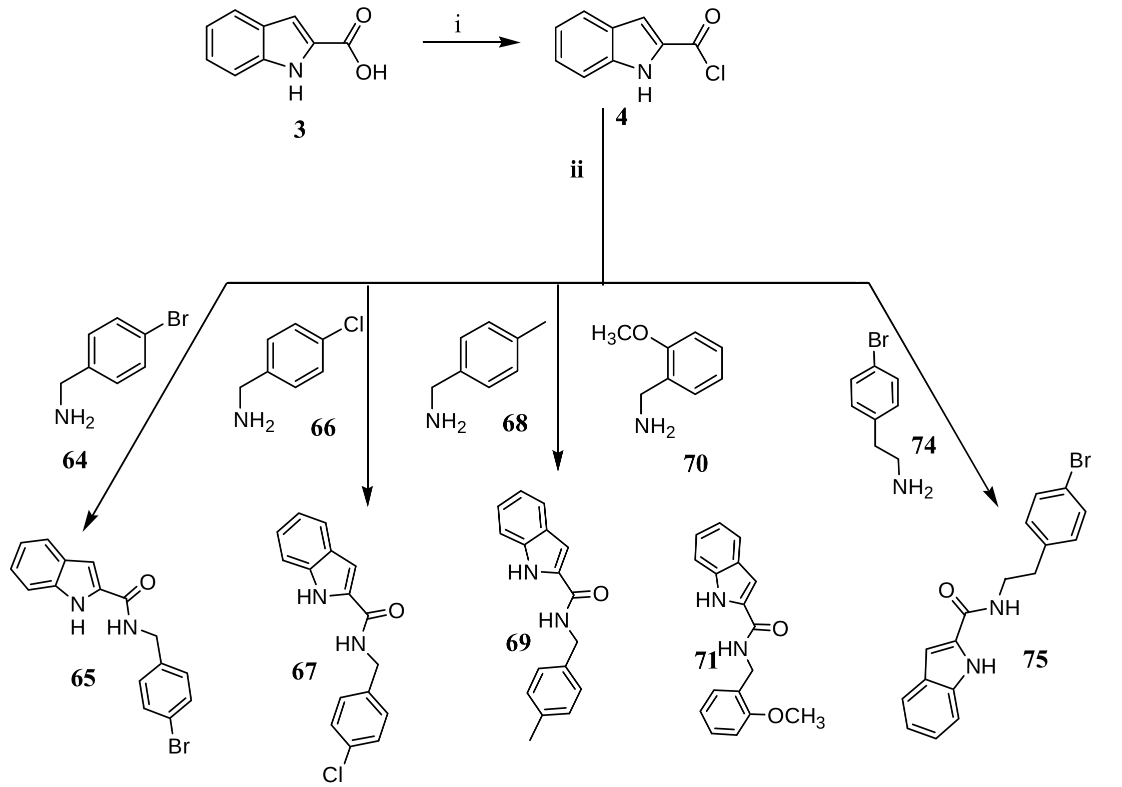


Scheme 12. Preparation of methyl-{3-[(1*H*-indole-2-yl-carbonyl)-amino] phenylacetate and methyl 3-{3-[(1*H*-indole-2-ylcarbonyl)-amino] phenylpropanoate (**61** and **63**). i: $SOCl_2$, 80 °C, methanol; ii: TEA, DMF, 120°C.

All these compounds were characterized by NMR, IR and MS and were consistent with expected positions/ peaks, except for compounds (**61** and **63**), that gave major side products. Selected derivatives (**41, 47, 51, 53, 57** and **63**) have been evaluated for their antihyperlipidemic, and all found to be inactive. These findings indicated again that, additional benzene ring is essential for activity.

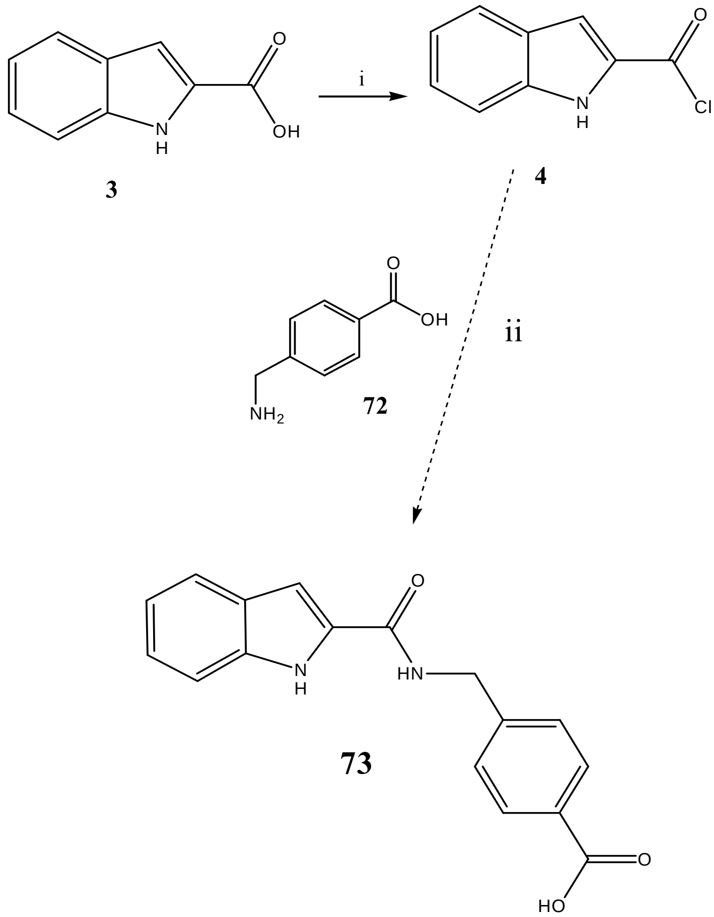
Unsubstituted indole benzyl and phenyl-ethyl carboxamides group (Series E):

The preparation of the target compounds (**65, 67, 69, 71** and **75**) was simply performed by stirring indole-2-carbonyl chloride (**4**), with the corresponding simple aromatic amines (**64, 66, 68, 70** and **74**) in $CHCl_3$ or DCM at 80 °C (Scheme 13).



Scheme 13. Preparation of indole-benzyl-2-carboxamide derivatives (**65, 67, 69** and **71**) and N-[2-(4-bromophenyl)ethyl]-1*H*-indole-2-carboxamide (**75**). i: $SOCl_2$, 70-80 °C, $CHCl_3$; ii: DCM, $CHCl_3$, 80 °C.

Target compound **(73)**, was synthesized by refluxing indole-2-carbonyl chloride (**4**), with 4-aminomethyl benzoic acid (**72**) at 80 °C, in DCM (Scheme 14). Apart from compound (**73**), all target compounds were obtained in good yield.

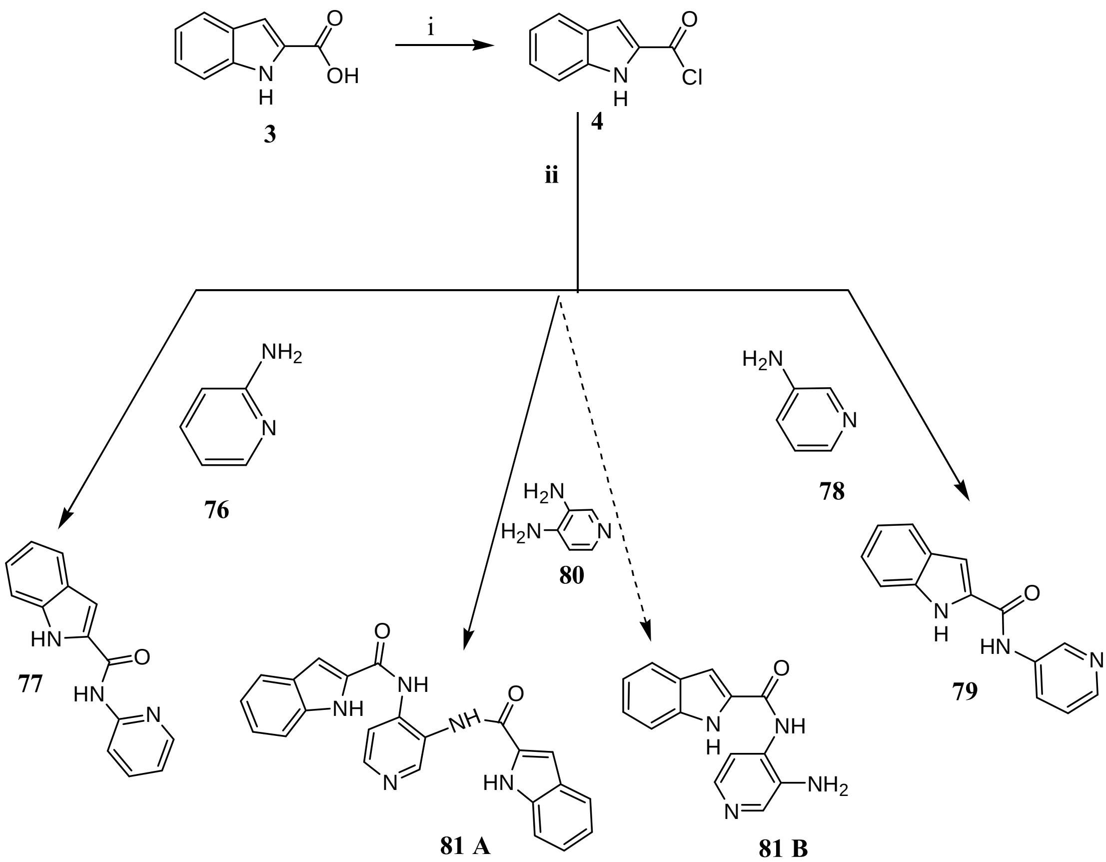


Scheme 14. Preparation of N-(4-amino-methyl benzoic acid)-1*H*-indole-2-carboxamide (**73**). i: $SOCl_2$, 70-80 °C, $CHCl_3$; ii: DCM, $NaOCH_3$, 80 °C.

All these compounds were characterized by NMR, IR and MS and were consistent with expected positions/peaks, except compound (**73**) due to low yield . Selected derivatives (**67** and **75**) have been evaluated for their antihyperlipidemic, and both found to be inactive as dyslipidemic compounds.

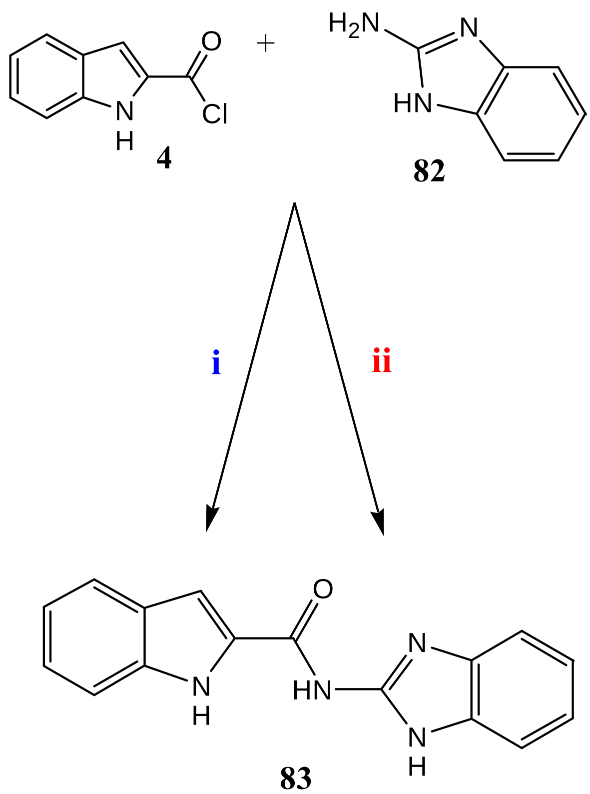
Unsubstituted indole heterocylic carboxamides group (Series F):

The preparation of the target compounds (**77, 79** and **81**) was performed by refluxing indole-2-carbonyl chloride (**4**), with the corresponding amines (**76, 78** and **80**) in DMF at 150 °C, for 2 days (Scheme 15). These compounds obtained in satisfactory yield. Target compound (**81**), found to form two amide linkage i.e form bisindole with 3,4-diaminopyridine (**80**), this was confirmed with NMR spectra and MS. The appearance of two carbonyl amide carbon, in addition to disappearance of the sharp singlet (2H) for NH_2 confirmed this structure. Further proof was obtained from MS and EA. Very small amount of **81B** were isolated from the column and confirmed by MS.



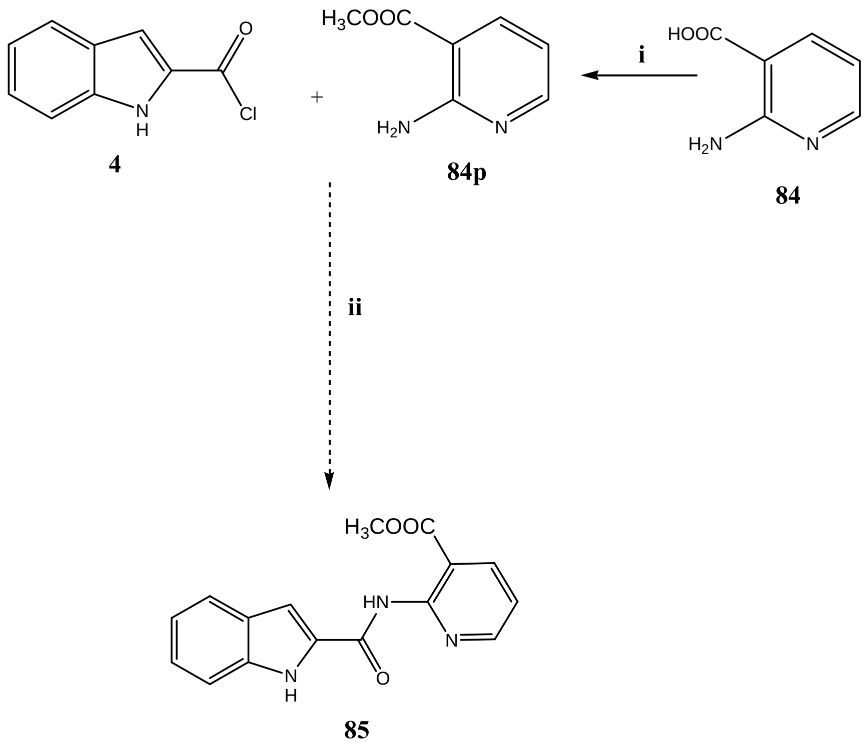
Scheme 15. Preparation of indole-pyridine-2-carboxamide derivatives (**77, 79** and **81**). i: $SOCl_2$, 70-80 °C, $CHCl_3$; ii: DMF, TEA 150 °C.

The target compound N-(1*H*-Benzimidazol-2-yl)-1*H*-indole-2-carboxamide (**83**), was synthesized using two procedures to optimize the yield (Scheme 16). The first procedure (a) involved refluxing indole-2-carbonyl chloride (**4**), with 2-aminobenzimidazole (**82)**, at 80 °C, in $CHCl_3$ using $NaOC_2H_5$ as the base. Which found to produce the target (**83**) but in very low yield. Alternatively (b), involved indole-2-carbonyl chloride (**4**) which was refluxed with 2- aminbenzimidazole (**82)**, at 150 °C, in DMF in the presence of TEA, furnished higher yield (15%). More attempts were made to produce the target (**83**) with better yield, but with no success; such as using different solvents (DMSO, 1,4-dioxane, $CHCl_3$), bases (pyridine, tertiary butoxide) and different temperatures.



Scheme 16. Preparation of N-(1*H*-Benzimidazol-2-yl)-1*H*-indole-2-carboxamide (**83**). i: $CHCl_3$, TEA, 80 °C; ii: DMF, TEA, 150 °C.

The last target of this series methyl 2-[(1*H*–indole–2–ylcarbonyl)amino] nicotinate (**85**), was successfully prepared, when converted Ortho-amino-nicotinic acid (**84**) to the ester counterpart methyl-2-amino nicotinate (**84p**), which was then treated with indole-2-carbonyl chloride (**4**) in DMF in the presence of TEA at 120 °C for 2 days, (Scheme 17). Low amount hindered the hydrolysis to (**85$_{acid}$**). No further attempts were carried out tomprepare (**85**), since it was found to be inactive.



Scheme 17. Preparation of N-(1*H*-Benzimidazol-2-yl)-1*H*-indole-2-carboxamide (**83**). i: $SOCl_2$, methanol, RT; ii: DMF, TEA, 120 °C.

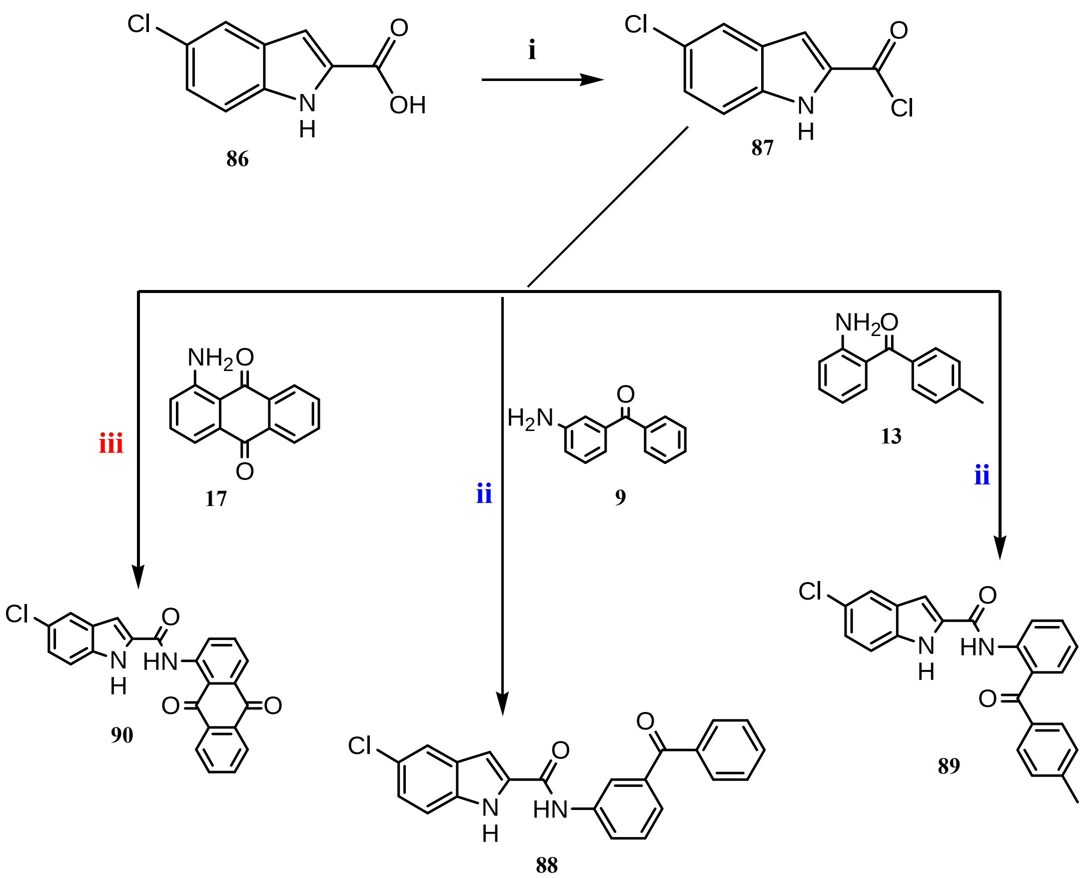
All these compounds were characterized by NMR, IR and MS and were consistent with expected positions/ peaks. Selected derivatives (**79, 83** and **85**) have been evaluated for their antihyperlipidemic, and all found to be inactive.

The evaluated dyslipidemic activity of series (C, D, E and F), implicated the importance of the lipophilicity of the amine groups, and the carbonyl linkage or its isoster as imine group as in compound (**38**) between the two phenyl groups in the targets.

Substituted indole carboxamides group (Series G):

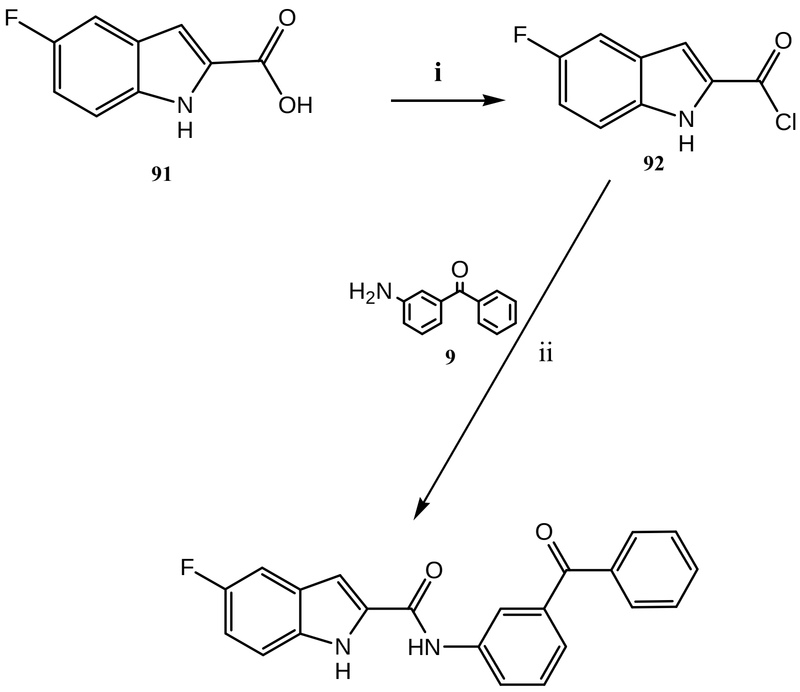
A. Five substituted indole derivatives:

A successful attempts were achieved to synthesize substituted 5-chloroindole derivatives, such as N-[3-benzoylphenyl]-5-chloro-1*H*-indole-2-carboxamide (**88**) and N-[2-(4-toloylphenyl)]-5-chloro-1H-indole-2-carboxamide (**89**) (Scheme 18). This procedure involved the use of 5-chloroIndole-2-carbonyl chloride (**87**), which was produced from 5-chloroindole-2-carboxylic acid (**86**) by acylation with thionyl chloride in DCM at 70-80 °C. Next, the corresponding benzophenone amine derivatives (**9, 13**) were added in the presence of acetonitrile and toluene at 90-110 °C. The precipitated products were filtered and recrystallized to afford pure target compounds (**88** and **89**). For the target compound N-(9,10-dihydro-9,10-dioxoanthracen-1-yl)-5-chloro-1*H* indole- 2-carboxamide (**90**), the procedure that gave high yield from previous anthraquinone methods was adopted, which was based on fusion at high temperature. 5-chloroIndole-2-carbonyl chloride (**87**) was thoroughly mixed with 1-aminoanthraquinone (**17**), refluxed at 120 °C for 18 hrs in air condenser, then 1,4-dioxane (15 mL) was added followed by stirring for additional 24 hrs at RT. The products were filtered and recrystalized from chloroform/methanol to give the desired compound (**90**) (Scheme 18).



Scheme 18. Preparation of N-[3-benzoylphenyl]-5-chloro-1*H*-indole-2-carboxamide (**88**), N-[2-(4-toloylphenyl)]-5-chloro-1H-indole-2-carboxamide (**89**) and N-(9,10-dihydro-9,10-dioxoanthracen-1-yl)-5-chloro-1*H* indole- 2-carboxamide (**90**). i: $SOCl_2$, 70-80 °C, $CHCl_3$; ii: acetonitrile, toluene, 90-110 °C; iii: refluxed at 120 °C .

Another target compound N-(3-Benzoylphenyl)-5-fluoro-1H-indole-2-carboxamide (**93**) (Scheme 19), was prepared by treating 5-fluoroindole-2-acylchloride (**92**) with 3-aminobenzophenone (**9**), in the presence of TEA in $CHCl_3$. The mixture was filtered and purified by recrystallization (Shattat *et al.*, 2010).



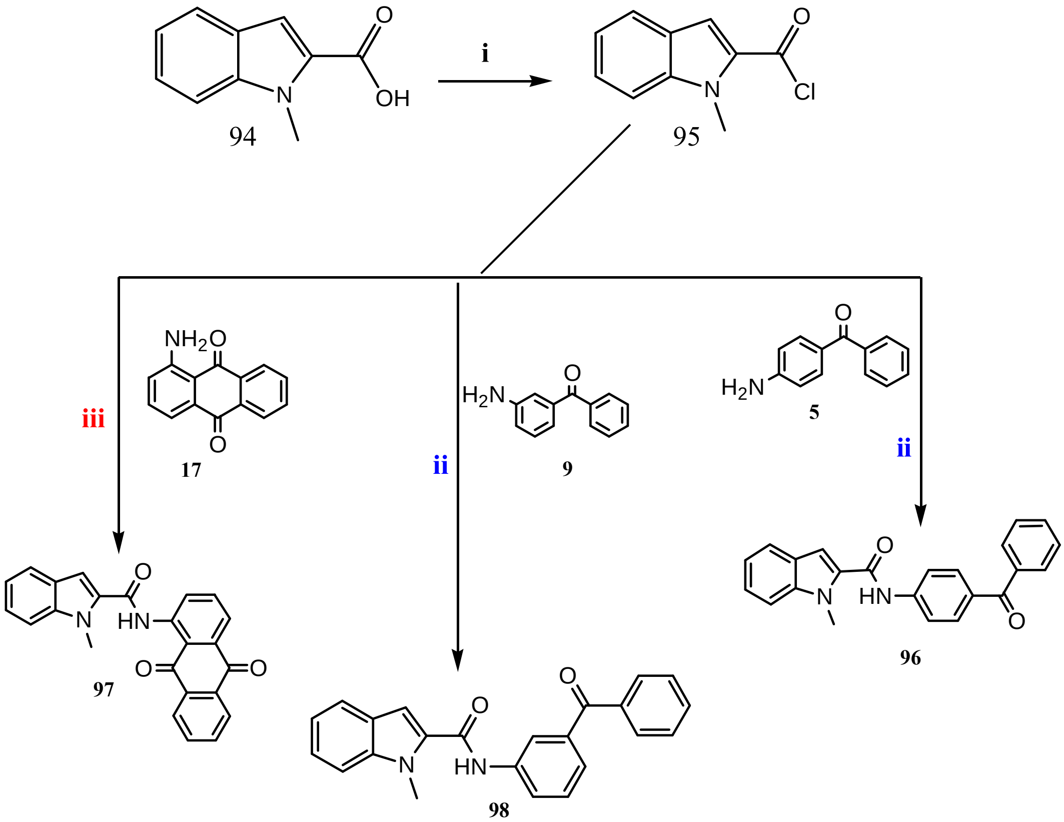
Scheme 19. Preparation of N-(3-Benzoylphenyl)-5-fluoro-1H-indole-2-carboxamide (**93**). i: $SOCl_2$, 90-100 °C; ii: TEA, $CHCl_3$, 60-70 °C (Shattat *et al.*, 2010).

.

B. N-substituted indole derivatives:

The target compounds N-[4-benzoylphenyl] -1- methyl -1*H*- indole- 2-carboxamide (**96**) and N- [3-benzoylphenyl] -1- methyl- 1*H*- indole- 2-carboxamide (**98**) (Scheme 20), were produced using N-methyl indole-2-carbonyl chloride (**95**), which was obtained from N-methyl indole-2-carboxylic acid (**94**), by acylation with thionyl chloride in $CHCl_3$ at 80 °C. Next, the corresponding benzophenone amine derivatives were added in the presence of $NaOC_2H_5$ in $CHCl_3$ at 100 °C. The precipitated products were filtered and recrystallized to afford pure target compounds (**96** and **98**). The target compound N-(9,10-dihydro-9,10-dioxoanthracen-1-yl)-1-methyl-1*H* indole- 2-carboxamide (**97**), was synthesized, based on fusion at high temperature. N-methyl indole-2-carbonyl chloride (**95**), was thoroughly mixed with 1-

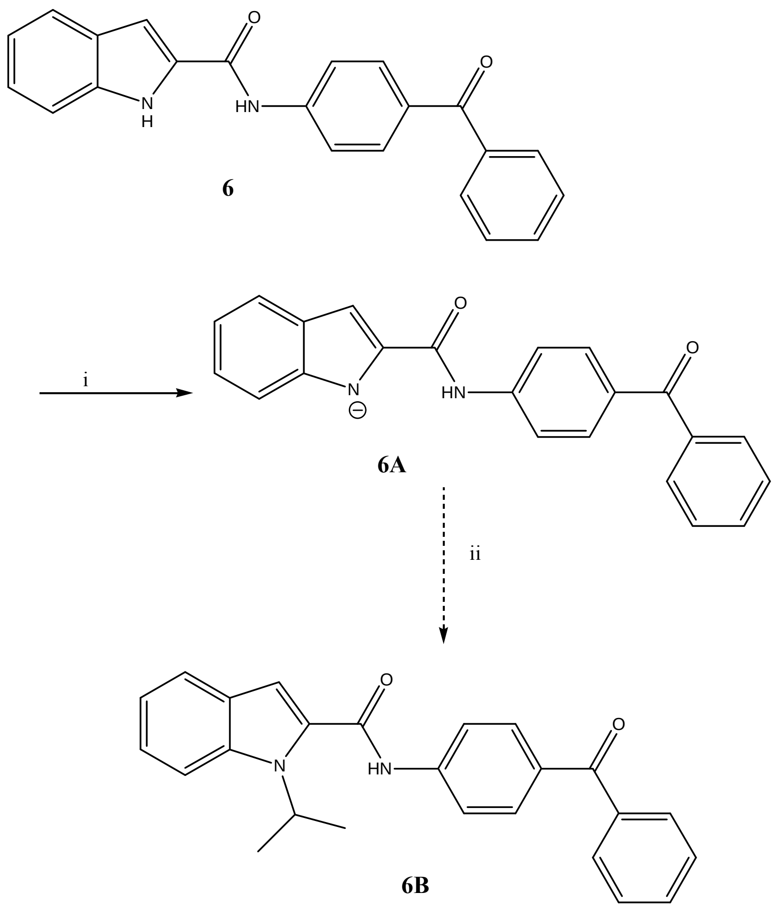
aminoanthraquinone (**17**), refluxed at 120 °C for 18 hrs in air condenser, then 1,4-dioxane (15 mL) was added followed by stirring for additional 24 hrs at RT. The mixture was filtered and recrystalized from chloroform/methanol to give the desired compound (**95**) (Scheme 19).



Scheme 20. Preparation of N-[4-benzoylphenyl]-1-methyl-1*H*-indole-2-carboxamide (**96**), N-[3-benzoylphenyl]-1-methyl-1*H*-indole-2-carboxamide (**98**) and N-(9,10-dihydro-9,10-dioxoanthracen-1-yl)-1-methyl-1*H* indole- 2-carboxamide (**97**). i: $SOCl_2$, 80 °C, $CHCl_3$; ii: $NaOC_2H_5$, 100 °C; iii: refluxed at 120 °C.

An attempt was carried out to prepare N-[4-benzoylphenyl]-1-isopropyl-1*H*-indole-2-carboxamide (**6B**) using sodium hydride (NaH) (Scheme 21). N-[4-benzoylphenyl]-1*H*-indole-2-carboxamide (**6**), was treated with excess NaH in dry DMSO and stirred for 30 minutes on an ice bath, followed by adding 2-iodopropane and mixing for 12 hrs at RT. The mixture was extracted with

ether, to give a very low amount of (**6B**). No further attempt was conducted to prepare the compound, since N-methyl indole was found to be inactive, which implicated the importance of having free N-H indole for anti-dyslipidemic activity.



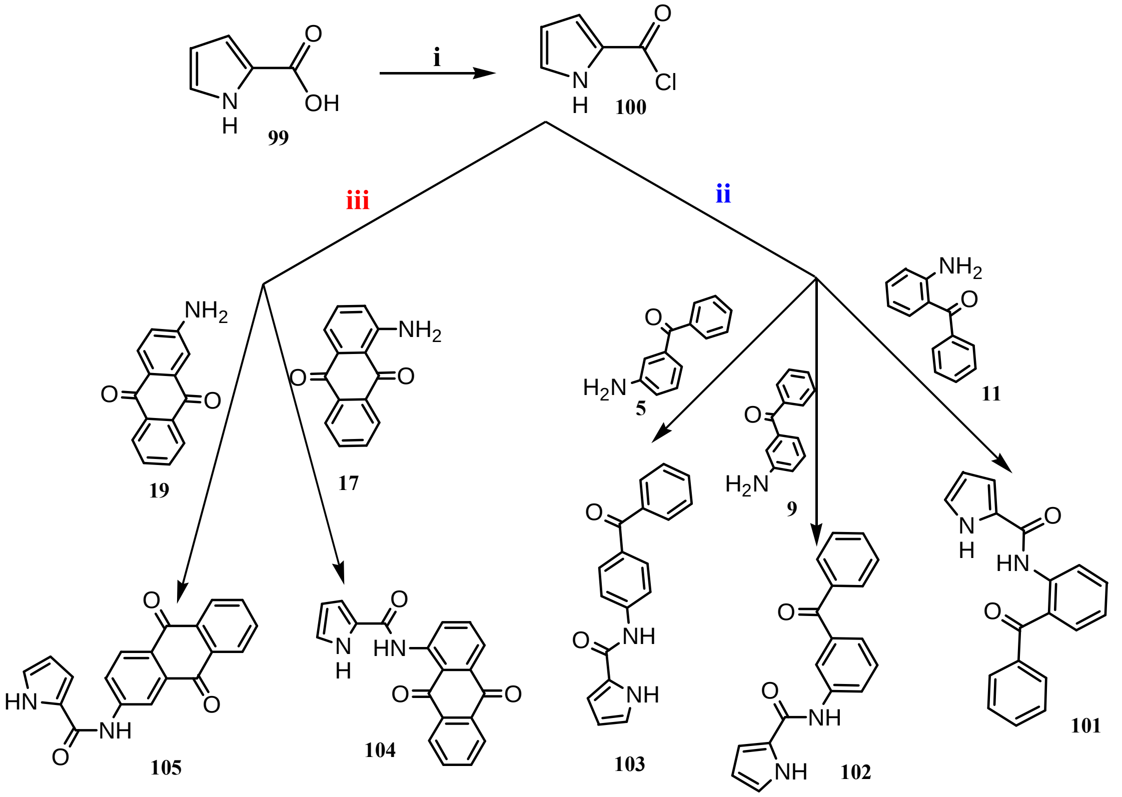
Scheme 21. Preparation of N-[4-benzoylphenyl]-1-isopropyl-1*H*-indole-2-carboxamide (**6B**). i: NaH, DMSO, 0°C; ii: 2-iodopropane, 12 hrs, RT.

All these compounds were characterized by NMR, IR and MS and were consistent with expected positions/ peaks. Selected targets (**88, 90, 93, 96** and **97**) have been evaluated for their antihyperlipidemic, compounds (**88, 90** and **93**) were active, but compounds (**96** and **97**) were not active. This implicated the importance of (NH) in the indole moiety for H-bonding with the receptor.

Removal of benzene group from indole carboxamides group (Series H):

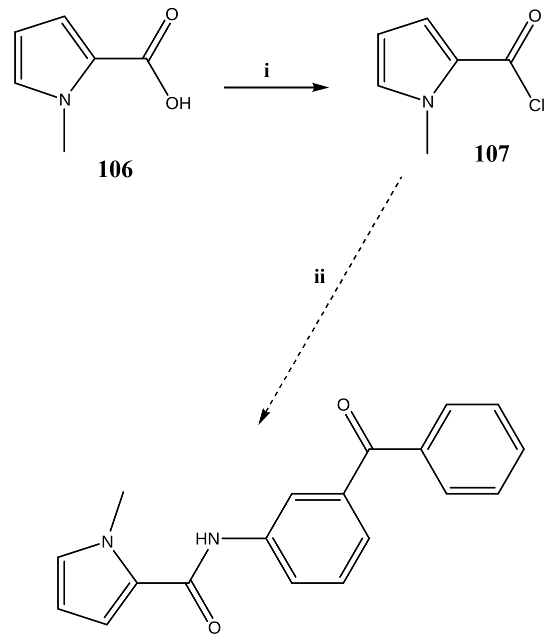
A. Pyrrole 2-carboxamide derivatives:

A number of attempts were carried out to synthesize π-excessive 5-membered ring isostere system derivative to establish SARs. Substituted pyrrole-2-carboxamide derivatives, such as N-[2-benzoylphenyl]-1*H*-pyrrole-2-carboxamide (**101**), N-[3-benzoylphenyl]-1*H*-pyrrole-2-carboxamide (**102**) and N-[4-benzoyl-phenyl]-1*H*-pyrrole-2-carboxamide (**103**) were successfully prepared (Scheme 22). This procedure involved the use of pyrrole-2-carbonyl chloride (**100**), which was produced from pyrrole-2-carboxylic acid (**101**) by acylation with thionyl chloride in DCM at 50 °C. Next, the corresponding benzophenone amine derivatives were added in the presence of acetonitrile and toluene or 1,4-dioxane at 90-110 °C. The precipitated products were filtered and recrystallized to afford pure target compounds (**101, 102** and **103**). For the target compounds N-(9,10-dihydro-9,10-dioxoanthracen-1-yl)-1*H* pyrrole - 2-carboxamide (**104**) and N-(9,10-dihydro-9,10-dioxoanthracen-2-yl)-1*H* pyrrole - 2-carboxamide (**105**). The procedure was based on fusion at high temperature. Pyrrole-2-carbonyl chloride (**100**), was thoroughly mixed with aminoanthraquinone derivatives (**17** and **19**), refluxed at 90 °C for 18 hrs in air condenser, then 1,4-dioxane (15 mL) was added followed by stirring for additional 24 hrs at RT. The products were filtered and recrystalized from chloroform/methanol to give the desired compounds (**104** and **105**) (Scheme 22).



Scheme 22. Preparation of N-[2-benzoylphenyl]-1*H*-pyrrole-2-carboxamide (**101**), N-[3-benzoylphenyl]-1*H*-pyrrole-2-carboxamide (**102**), N-[4-benzoyl-phenyl]-1*H*-pyrrole-2-carboxamide (**103**) and N-(9,10-dihydro-9,10-dioxoanthracen-1-yl)-1*H* pyrrole - 2-carboxamide (**104**) and N-(9,10-dihydro-9,10-dioxoanthracen-2-yl)-1*H* pyrrole - 2-carboxamide (**105**). i: $SOCl_2$, 50 °C, DCM; ii: acetonitrile, toluene or dioxane, 90-120 °C; iii: refluxed at 90 °C.

The target compound N-[3-benzoylphenyl]-1-methyl-1*H*-pyrrole-2-carboxamide (**108**) (Scheme 23), was produced using N-methyl-pyrrole-2-carbonyl chloride (**107**), which was obtained from N-methylpyrrole-2-carboxylic acid (**106**), by acylation with thionyl chloride in DCM at 50 °C. Next, 3-aminobenzophenone (**9**) was added in the presence of acetonitrile in toluene at 100 °C. The precipitated products were filtered and recrystallized to afford the target compound (**108**).

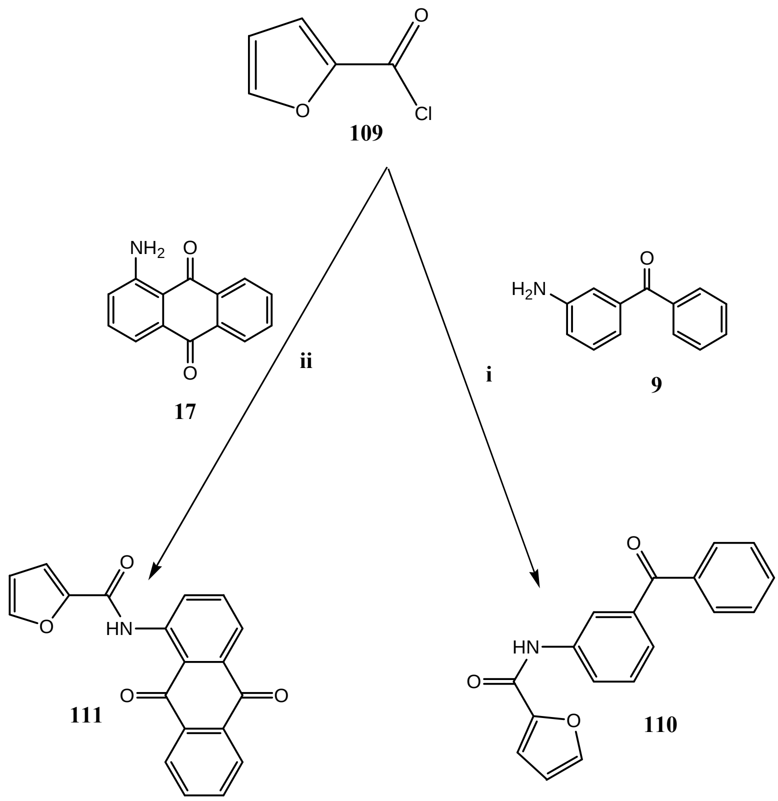


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Scheme 23. Preparation of N-[3-benzoylphenyl]-1-methyl-1*H*-pyrrole-2-carboxamide (**108**). i: $SOCl_2$, 50 °C, DCM; ii: acetonitrile, dioxane, 90 °C.

B. Furan 2-carboxamide derivatives:

The target compound N-(3-benzoylphenyl)-2-furamide (**110**) (Scheme 24), was synthesized by treating 3-aminobenzophenone (**9**), in acetonitrile and DCM at 80 °C. The precipitated products was filtered and recrystallized to afford a pure target compound (**110**). For the target compounds N-(9,10-dihydro-9,10-dioxoanthracen-1-yl)-2-furamide (**111**). The procedure was based on fusion at high temperature, 2-furonyl chloride (**109**), was thoroughly mixed with 1-aminoanthraquinone (**17**), refluxed at 120 °C for 18 hrs in air condenser, then 1,4-dioxane (15 mL) was added followed by stirring for additional 24 hrs at RT. The crude product was filtered and recrystalized to give the desired compounds (**111**) (Scheme 24).



Scheme 24. Preparation of N-(3-benzoylphenyl)-2-furamide (**110**) and N-(9,10-dihydro-9,10-dioxoanthracen-1-yl)-2-furamide (**111**). i: acetonitrile, DCM, 80 °C; iii: dioxane, RT.

All these compounds were characterized by NMR, IR and MS and were consistent with expected positions/ peaks. Selected targets (**101, 103, 104, 108, 110** and **111**) have been evaluated for their antihyperlipidemic. Compounds (**103, 104, 110** and **111**) were active, but compounds (**100** and **108**) were inactive. This implicated that benzene ring of the indole moiety is not crucial for activity and confirmed the previous conclusion that (NH) should be free for H-bonding with the receptor. Similar to previous findings, compound (**100**) was inactive, indicating that for the compound to be active, the structure should be extended as seen in compounds (**103** and **6**)

8.2. Biological Studies:

8.2.1. Induction of Hyperlipidemia by Triton WR-1339:

Triton WR-1339 has been widely used to block clearance of triglyceride-rich lipoproteins to induce acute hyperlipidemia in several animals. This model is widely used for a number of different aims particularly, in rats it has been used for screening natural or chemical hypolipidemic drugs. A single parenteral administration of Triton WR-1339 to adult rats produces hyperlipidemia in which cholesterol, triglycerides and phospholipids increase to a maximum in about 20 hrs and decrease thereafter (Harnafi, *et al*., 2008).

After 8 hours of the induction of hyperlipidemia with Triton WR 1339 at the dose of 250 mg/kg i.p., there was a marked increase in total cholesterol ($p < 0.0001$) and triglyceride concentrations ($p < 0.0001$).

The plasma cholesterol increased by more than two folds higher than the normal levels and triglycerides by more than 11 times (Figure 30). A significant increase in LDL-levels ($p < 0.0001$) were also observed in the rats treated with Triton alone. Along with all the above changes, Triton caused a drastic reduction ($p < 0.0001$) in HDL-levels, in the hyperlipidemic control group (HG) when compared to normal control group (CG). DMSO control was carried out producing no significant diffenrence from CG (Table 2).

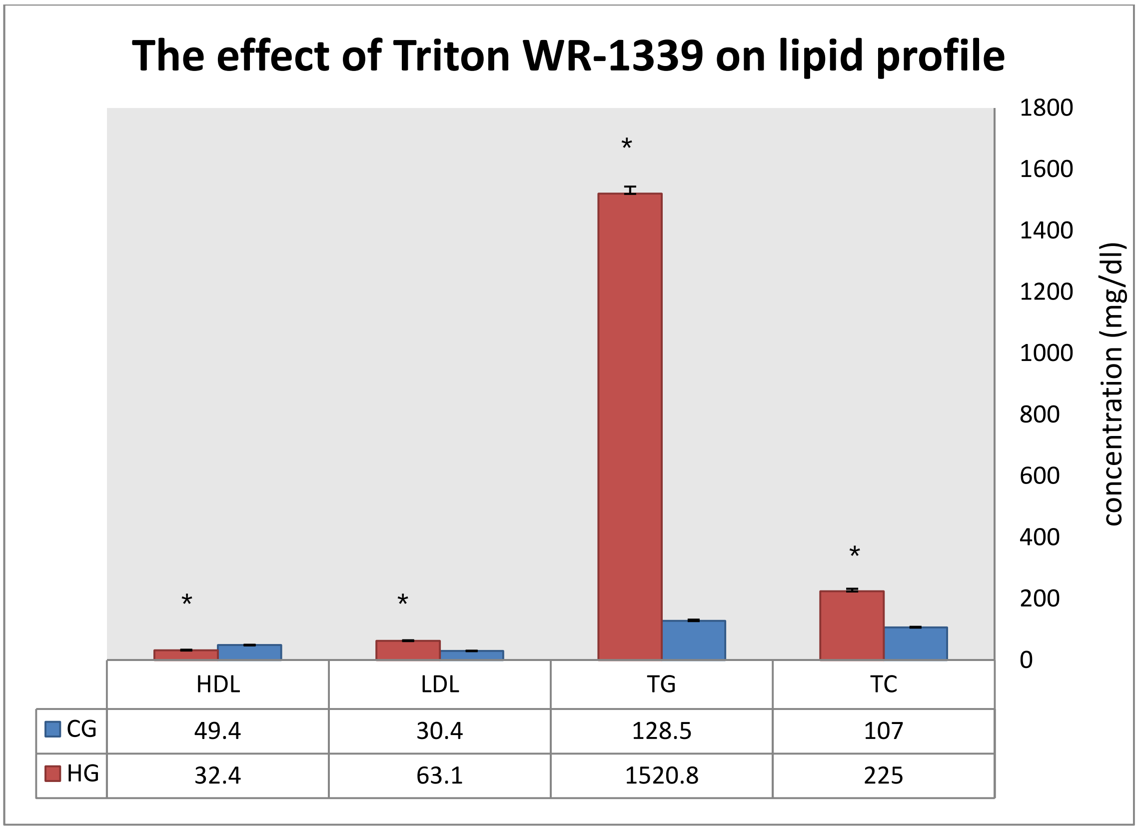


Figure 30. Effect of Triton WR-1339 on lipid profile after 8 hrs. Values are means ± SEM from six rats in each group. CG: control group; HG: hyperlipidemic control group; TC: total cholesterol; TG: triglyceride; HDL-C: high density lipoprotein-cholesterol; LDL-C: low-density lipoprotein-cholesterol. HG is compared to CG.
*$p < 0.0001$.

8.2.2. Dose selection:

The experiment to determine the lethal dose (LD_{50}) of the target compound (C6) was designed by using six different concentrations of the sample (200, 400, 600, 800, 1000 and 1200 mg/kg), which were orally administered to each group of six rats (Table 1). After oral administration, the survival rate were carefully and daily recorded over a period of 72 hours. The survival rate of the rat was calculated as (number of live rats/ total number of rats tested) ×100% (Jia *et al*., 2001; Batina *et al*., 1997). Based on the above results the approximate LD_{50} was estimated to be about 800 mg/kg which is equivalent to ≈ 200 mg for a rat weighing 250g.

Table 1. An oral acute toxicity study for the target compound (**C6**).

Dose (mg/kg)	Number of rats	Survival rate %
200	6	100 %
400	6	100 %
600	6	83 %
800	6	50 %
1000	6	17 %
1200	6	0 %

For the assessment of all the biological activities, a fixed dose of the target compounds was obtained during the acute toxicity studies and the concentration was 57 µM, the corresponding doses of the tested compounds in mg/kg are summarized in table (appendix II). The bezafibrate dose taken was 100 mg (276 µM) based on literature recommendations (Nakajima *et al*., 2009). The investigated compounds were dissolved in 4% dimethyl-sulfoxide (DMSO) for oral administration.

8.2.3. Effect of novel unsubstituted indole-benzophenone carboxamides group (Series A) and BF on Rat Plasma Lipid Profile:

The plasma TC, TG, HDL-C and LDL-C levels of BF, and benzophenone compounds treated rats 8 hrs after Triton administration are shown in (Table **2**, Fig. **31, 32, 33, 34** and **35**), respectively.

Cholesterol levels:

The results presented in Table 2 indicated that there was a significant decrease (21% $P<0.001$) in cholesterol levels after **C6** administration. However, no significant

differences in plasma total cholesterol levels between any treated groups (**BF**, compounds **C10, C14, C8** and **C16**) were observed in comparison to HG-treated rats.

Triglyceride levels:

The results presented in Table 2 indicated that the elevated plasma TG levels produced by Triton WR-1339 administration were significantly suppressed (62%, 71% and 71% $p < 0.0001$) in compounds **BF, C6** and **C10** respectively with respect to hyperlipidemic control HG. While no significant difference in TG levels were observed in compounds **C14, C8** and **C15** compared to HG-treated rats.

HDL-C levels:

HDL-C levels were significantly increased after 8 h of Triton administration (63%, 59% and 28% $p<0.0001$) in compounds **C6, C10** and **BF** respectively compared to HG (Table 2). In contrast there were no significant difference observed, in HDL-C levels, in compounds **C14, C8** and **C16** compared to HG-treated rats.

LDL levels:

The elevated plasma LDL levels produced by Triton WR-1339 administration were significantly suppressed (27% $p < 0.0001$) in compound **C6** and (26% $p < 0.001$) in compound **C10** with respect to hyperlipidemic control HG. While no significant difference in LDL levels were observed in compounds **C14, C8, C16** and **BF** compared to HG-treated rats.

In summary, a significant decrease in TG and LDL and an increase in HDL plasma levels were observed with compounds **C6** and **C10**. In addition, compound **C6** showed a significant reduction in TC levels too.

Table 2. The effect of the novel unsubstituted indole benzophenone carboxamide on plasma lipid levels in Triton WR-1339-induced hyperlipemic rats after 8 hrs.

Lipid profile	TC (mg/dl)	TG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
CG	105 ± 1.70	126.9 ± 3.32	48.9 ± 1.29	31.1 ± 1.1
HG	225 ± 7.78 [c]	1520.8 ± 22.86 [c]	32.4 ± 2.0 [c]	63.1 ± 2.15 [c]
C6	178.3 ± 2.25 [b]	435 ± 14.5 [c]	52.1 ± 0.84 [c]	46.4 ± 1.6 [c]
C10	234 ± 10.04	440 ± 25.92 [c]	51.3 ± 2.69 [c]	46.7 ± 2.66 [b]
C14	244 ± 18.29	1522.3 ± 7.73	28.5 ± 2.13	66.3 ± 1.11
C8	277.2 ± 15.01 [a]	1521 ± 18	29.98 ± 1.37	60.9 ± 1.89
C16	227.67 ± 7.77	1523.3 ± 13.15	29.8 ± 1.75	59.2 ± 2.73
BF	219.2 ± 6.18	579 ± 18.4 [c]	42.3 ± 1.24 [c]	62.3 ± 2.08
PCG	105 ± 1.66	129 ± 2.87	49.1 ± 0.79	31.1 ± 0.95

Values are means ± SEM from six rats in each group. CG: normal control group; PCG: positive control group, 4% DMSO; HG: hyperlipidemic +4% DMSO control group; C6: compound C6+4% DMSO; C10: compound C10+4% DMSO; C14: compound C14+4% DMSO; C8: compound C8+4% DMSO; C16: compound C16+4% DMSO; BF: bezafibrate+4% DMSO; TC: total cholesterol; TG: triglyceride; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol. HG is compared to CG.
C6, C10, C14, C8 , C16 and BF are compared with HG.
[a] $p<0.01$, [b] $p<0.001$, [c] $p<0.0001$

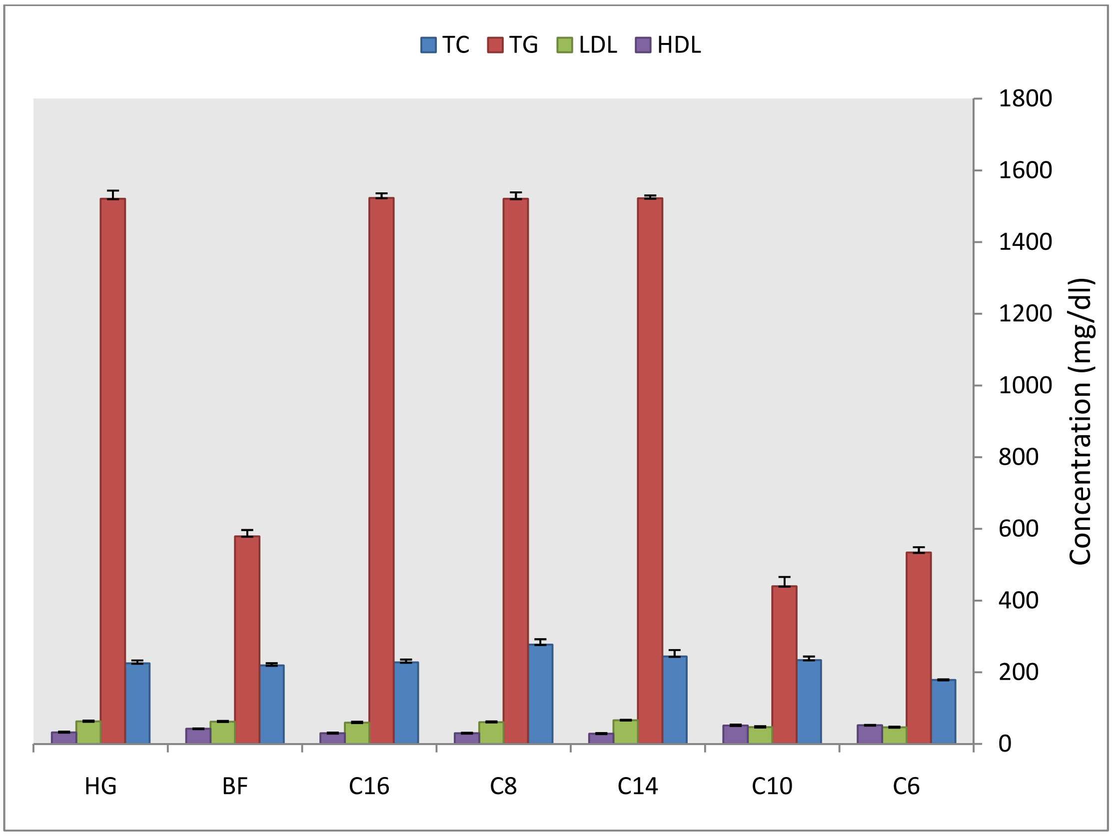


Figure 31. Effect of the novel indole benzophenone carboxamide compounds on lipid profile (mg/dl) after 8 hrs of Triton administration.

Values are means ± SEM from six rats in each group. CG: normal control group; HG: hyperlipidemic +4% DMSO control group; C6: compound C6+4% DMSO; C10: compound C10+4% DMSO; C14: compound C14+4% DMSO; C8: compound C8+4% DMSO; C16: compound C16+4% DMSO; BF: bezafibrate+4% DMSO; TC: total cholesterol; TG: triglyceride; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol. HG is compared to CG.

C6, C10, C14, C8 , C16 and BF are compared with HG.

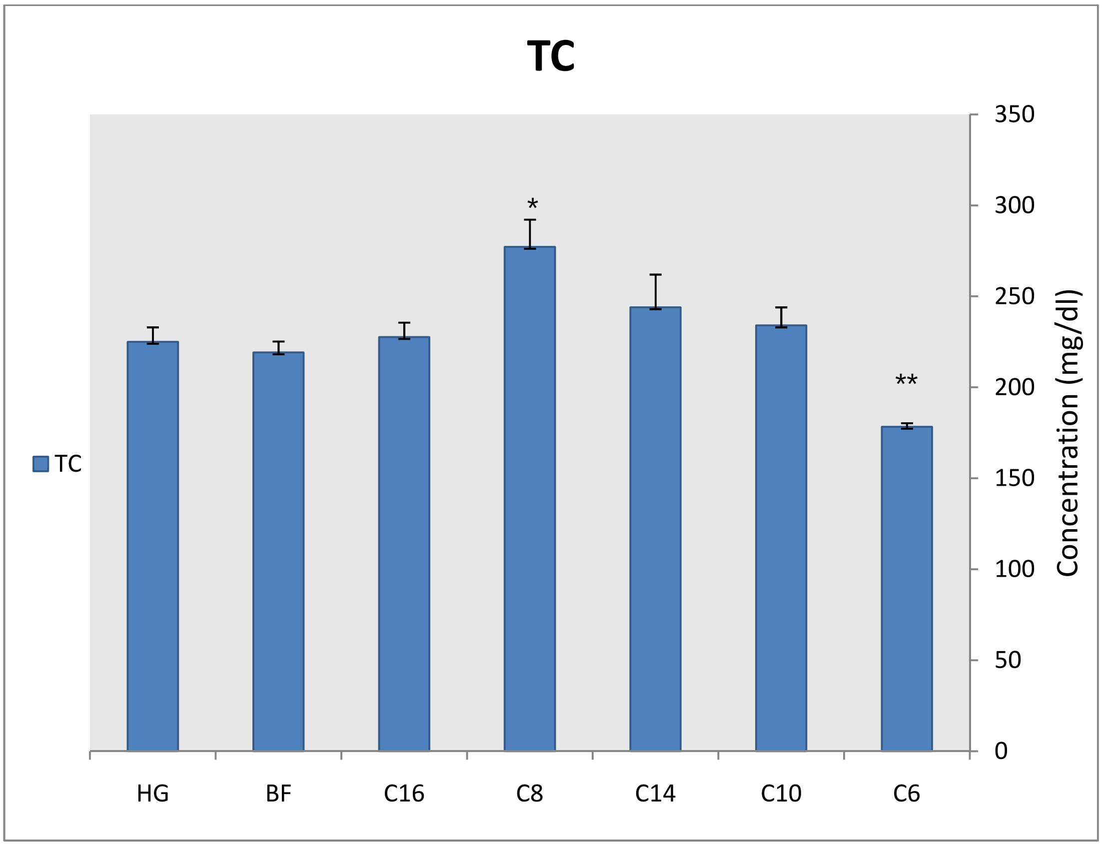


Figure 32. Effect of the novel indole benzophenone carboxamide compounds on total cholesterol (mg/dl) after 8 hrs of Triton administration.
Values are means ± SEM from six rats in each group. CG: normal control group; HG: hyperlipidemic +4% DMSO control group; C6: compound C6+4% DMSO; C10: compound C10+4% DMSO; C14: compound C14+4% DMSO; C8: compound C8+4% DMSO; C16: compound C16+4% DMSO; BF: bezafibrate+4% DMSO; TC: total cholesterol; TG: triglyceride; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol. HG is compared to CG.
C6, C10, C14, C8 , C16 and BF are compared with HG.
$^{*}p< 0.05$, $^{**}p<0.001$

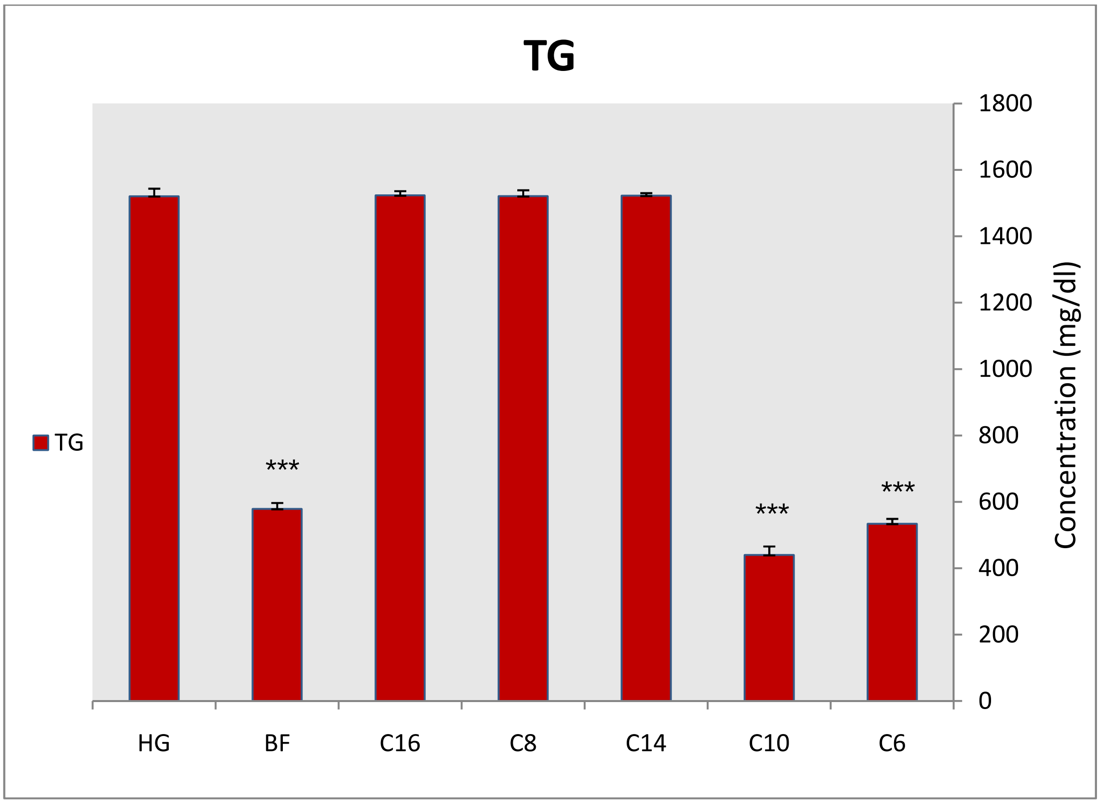


Figure 33. Effect of the novel indole benzophenone carboxamide compounds on Triglycerides (mg/dl) after 8 hrs of Triton administration.
Values are means ± SEM from six rats in each group. CG: normal control group; HG: hyperlipidemic +4% DMSO control group; C6: compound C6+4% DMSO; C10: compound C10+4% DMSO; C14: compound C14+4% DMSO; C8: compound C8+4% DMSO; C16: compound C16+4% DMSO; BF: bezafibrate+4% DMSO; TC: total cholesterol; TG: triglyceride; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol. HG is compared to CG.
C6, C10, C14, C8 , C16 and BF are compared with HG.
***p<0.0001

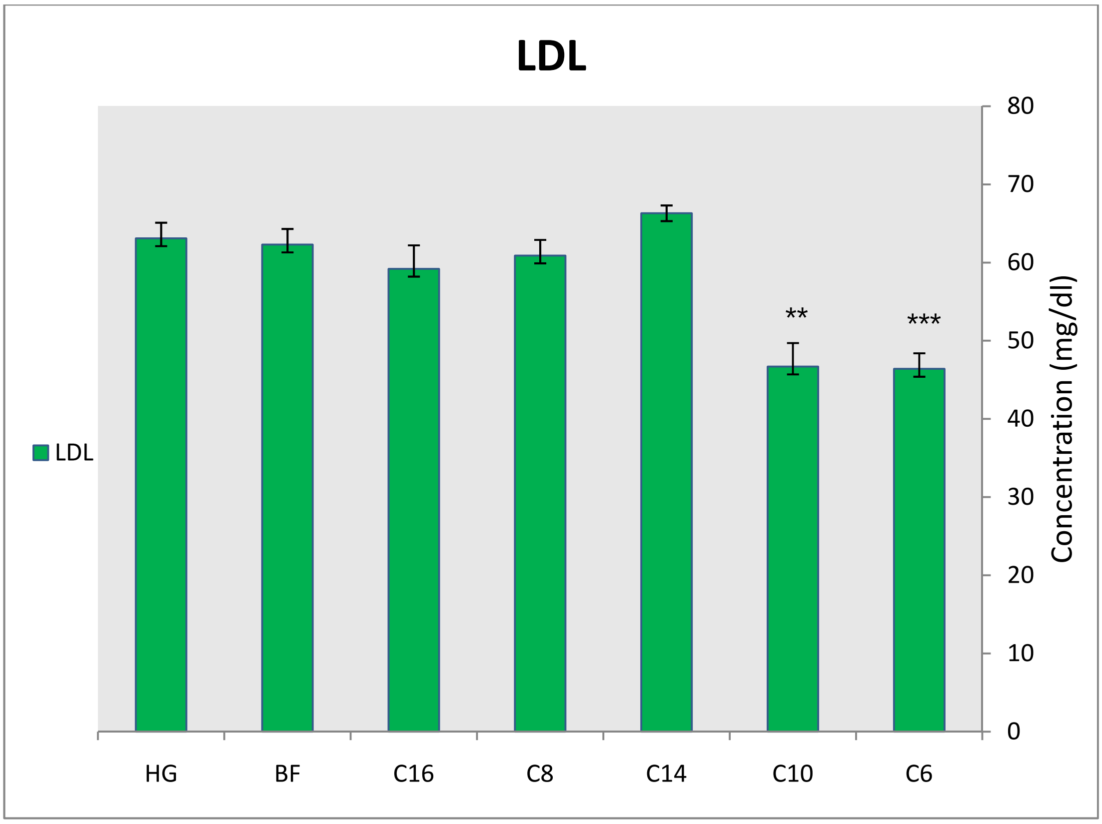


Figure 34. Effect of the novel indole benzophenone carboxamide compounds on low-density lipoproteins (mg/dl) after 8 hrs of Triton administration.

Values are means ± SEM from six rats in each group. CG: normal control group; HG: hyperlipidemic +4% DMSO control group; C6: compound C6+4% DMSO; C10: compound C10+4% DMSO; C14: compound C14+4% DMSO; C8: compound C8+4% DMSO; C16: compound C16+4% DMSO; BF: bezafibrate+4% DMSO; TC: total cholesterol; TG: triglyceride; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol. HG is compared to CG.

C6, C10, C14, C8 , C16 and BF are compared with HG.

$^{**}p<0.001$, $^{***}p<0.0001$

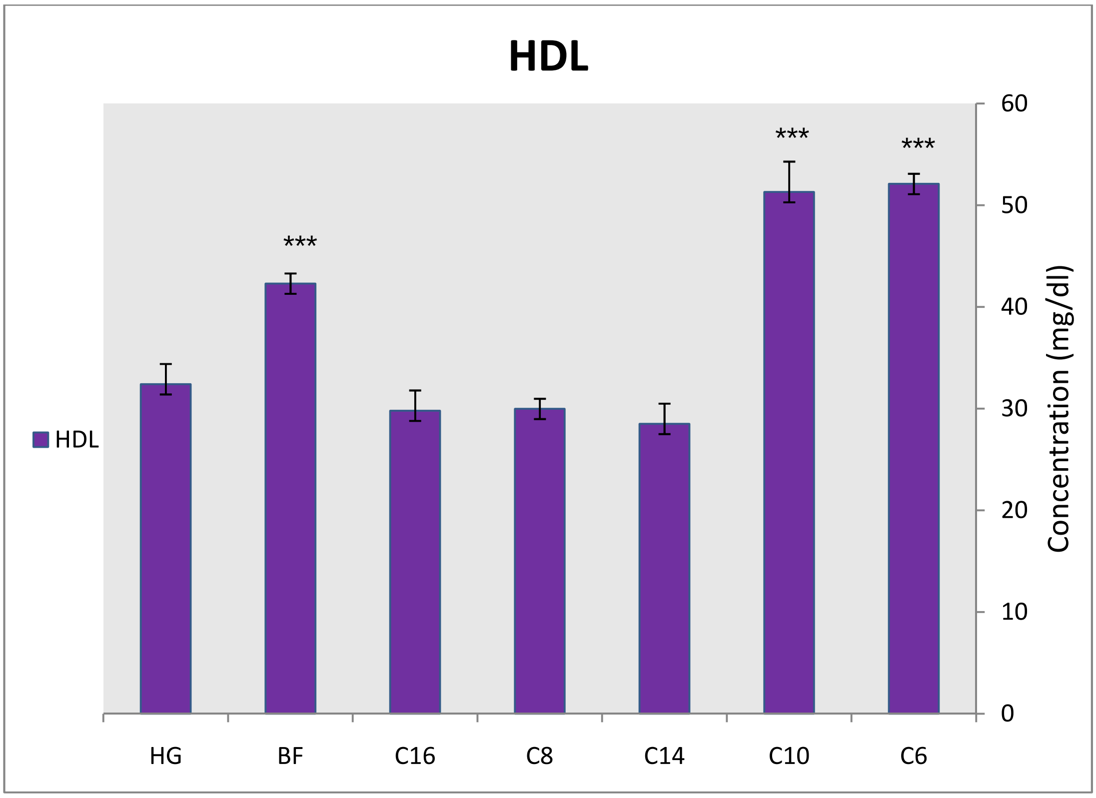


Figure 35. Effect of the novel indole benzophenone carboxamide compounds on high-density lipoproteins (mg/dl) after 8 hrs of Triton administration.
Values are means ± SEM from six rats in each group. CG: normal control group; HG: hyperlipidemic +4% DMSO control group; C6: compound C6+4% DMSO; C10: compound C10+4% DMSO; C14: compound C14+4% DMSO; C8: compound C8+4% DMSO; C16: compound C16+4% DMSO; BF: bezafibrate+4% DMSO; TC: total cholesterol; TG: triglyceride; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol. HG is compared to CG.
C6, C10, C14, C8 , C16 and BF are compared with HG.
$^{***}p<0.0001$

8.2.4. Effect of novel unsubstituted indole-anthraquinone carboxamides group (Series B) and BF on Rat Plasma Lipid Profile:

The plasma TC, TG, HDL-C and LDL-C levels of BF, and unsubstituted indole-anthraquinone carboxamide compounds treated rats 8 hrs after Triton administration are shown in (Table **3**, Fig. **36, 37, 38, 39** and **40**), respectively.

Cholesterol levels:

The results presented in Table **3** indicated that the cholesterol levels decreased (20% $P<0.001$) in compound **C18**, (20%, 17% $p< 0.0001$) in compound **C24** and **C26** respectively with respect to hyperlipidemic control HG. However, no significant difference in plasma total cholesterol levels were observed in compounds **C20, C22** and **BF** compared to HG-treated rats.

Triglyceride levels:

The results presented in Table **3** indicated that the elevated plasma TG levels produced by Triton WR-1339 administration were significantly suppressed (62%, 65%, 70%, 70% and 71% $p< 0.0001$) in compounds **BF, C18, C22, C24** and **C26** respectively with respect to HG-treated groups. While no significant difference in TG levels were observed in compound **C20** compared to HG-treated rats.

HDL-C levels:

HDL-C levels were significantly increased after 8 h of Triton administration (60% $p< 0.001$) in both compounds; **C24** and **C26**; and (62%, 62% and 28% $p<0.0001$) in compounds **C18, C22** and **BF** respectively compared to HG. While no significant difference in HDL-C levels were observed in **C20** (Table 3).

LDL levels:

The elevated plasma LDL levels produced by Triton WR-1339 administration were significantly suppressed (24% $p< 0.0001$) in compound **C18**, (28%, 29% and 27% p<

0.001) in compounds **C22, C24** and **C26** respectively with respect to HG-treated groups. While no significant difference in LDL levels were observed in compounds **C20** and **BF** compared to HG-treated rats.

In summary, a significant decrease in TG and LDL and an increase in HDL-C plasma levels were observed with compounds **C18, C22, C24** and **C26**. In addition, compounds **C18, C24** and **C26** showed a significant reduction in TC levels too.

Table 3. Effect of the novel unsubstituted indole-anthraquinone carboxamide on plasma lipid levels in Triton WR-1339-induced hyperlipidemic rats after 8 hrs.

Lipid profile	TC (mg/dl)	TG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
CG	106.7 ± 1.54	127.1 ± 2.95	49.1 ± 0.85	30.7 ± 1.11
HG	222.7 ± 6.71^{c}	1510.5 ± 21.7^{c}	31.2 ± 2.5^{c}	63.2 ± 2.44^{c}
C18	179 ± 5.43^{b}	529 ± 9.43^{c}	52.6 ± 1.35^{c}	48.1 ± 0.99^{c}
C22	228 ± 9.23	456 ± 23.23^{c}	52.4 ± 1.36^{c}	45.3 ± 2.4^{b}
C24	180 ± 1.85^{c}	452 ± 22^{c}	51.8 ± 1.62^{b}	44.9 ± 1.69^{b}
C26	187 ± 3.48^{c}	441 ± 31.56^{c}	51.7 ± 1.32^{b}	45.9 ± 1.16^{b}
C20	272 ± 15.23	1523 ± 21.72	28.3 ± 2.76	65.4 ± 1.18
BF	2117.8 ± 6.18	565 ± 18.4^{c}	43.1 ± 1.24^{c}	62.7 ± 2.08

Values are means ± SEM from six rats in each group. CG: normal control group; HG: hyperlipidemic +4% DMSO control group; C18: compound C18+4% DMSO; C22: compound C22+4% DMSO; C24: compound C24+4% DMSO; C26: compound C26+4% DMSO; C20: compound C20+4% DMSO; BF: bezafibrate+4% DMSO; TC: total cholesterol; TG: triglyceride; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol. HG is compared to CG.
C18, C22, C24, C26, C20 and BF are compared with HG.
^{b} $p<0.001$, ^{c} $p<0.0001$

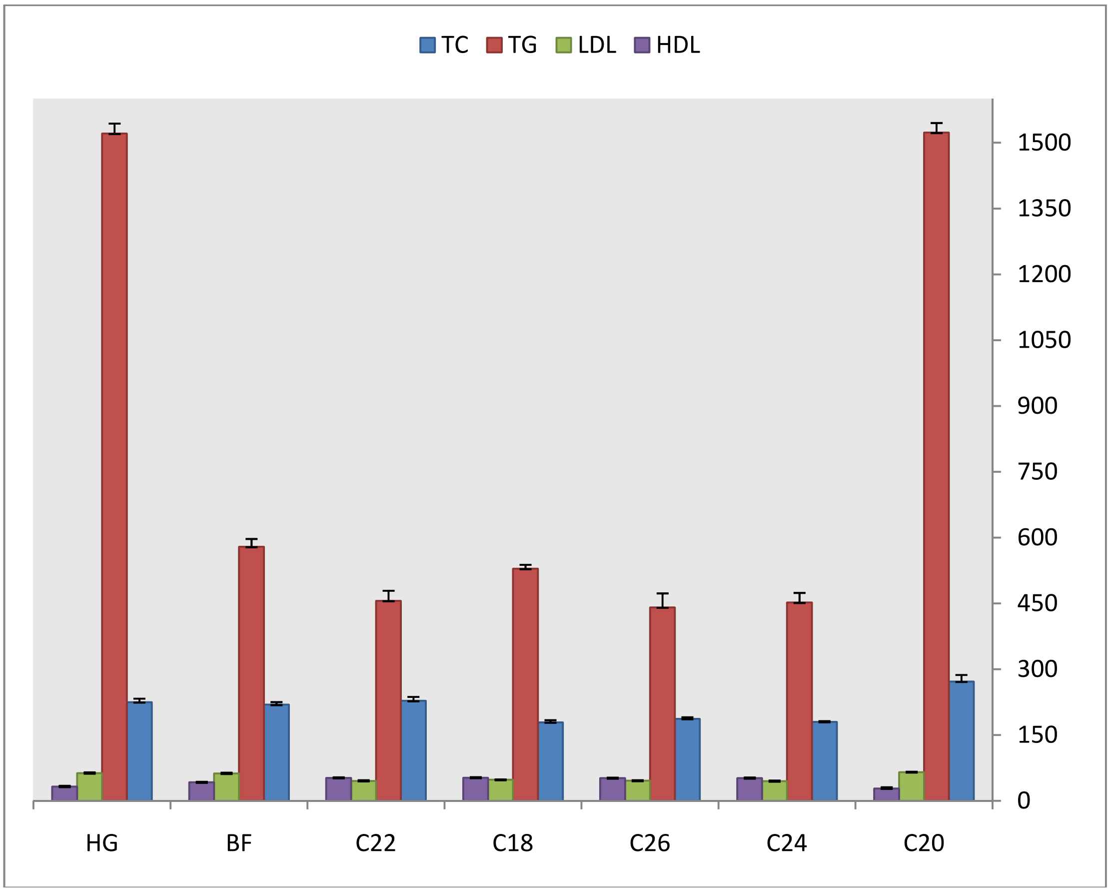


Figure 36. Effect of the novel unsubstituted indole-anthraquinone carboxamide on lipid profile (mg/dl) after 8 hrs of Triton administration.
Values are means ± SEM from six rats in each group. CG: normal control group; HG: hyperlipidemic +4% DMSO control group; C18: compound C18+4% DMSO; C22: compound C22+4% DMSO; C24: compound C24+4% DMSO; C26: compound C26+4% DMSO; C20: compound C20+4% DMSO; BF: bezafibrate+4% DMSO; TC: total cholesterol; TG: triglyceride; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol. HG is compared to CG.
C18, C22, C24, C26, C20 and BF are compared with HG.

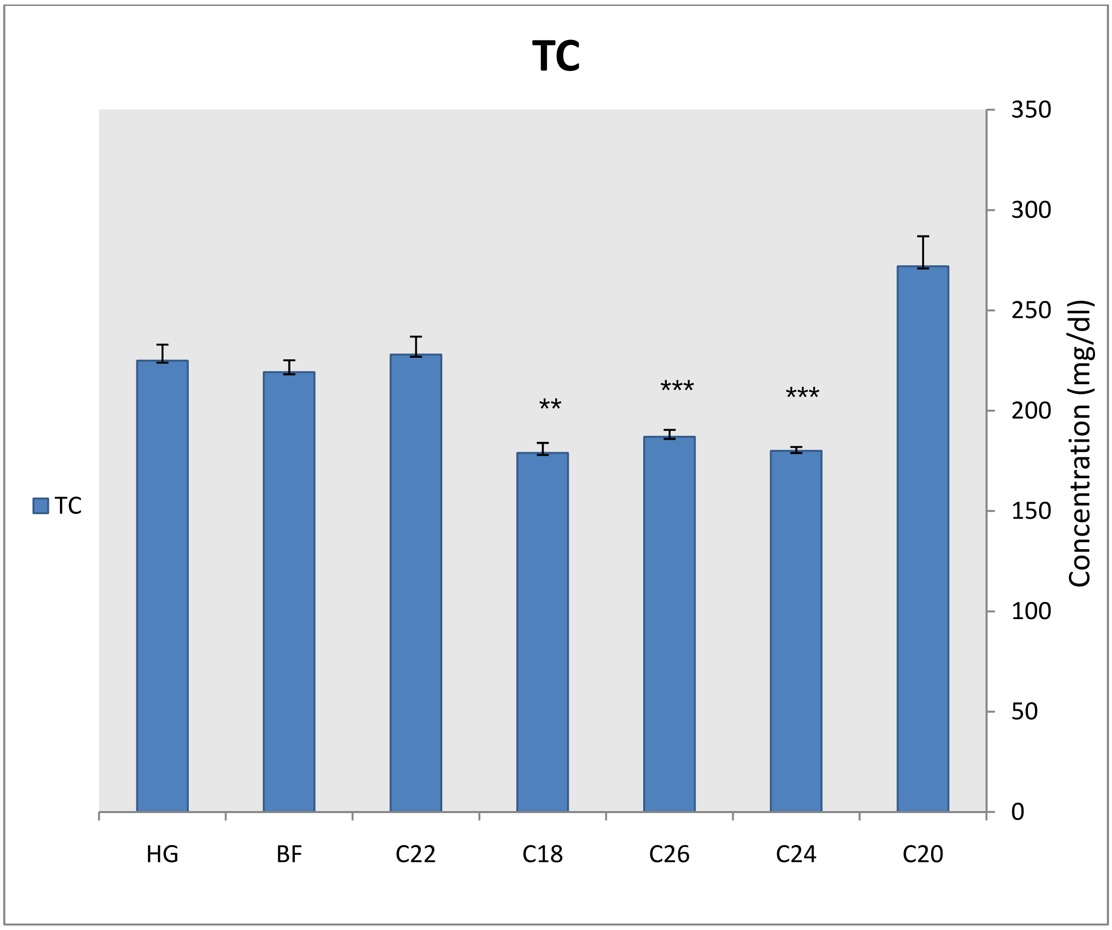


Figure 37. Effect of the novel unsubstituted indole-anthraquinone carboxamide on total cholesterol (mg/dl) after 8 hrs of Triton administration.

Values are means ± SEM from six rats in each group. CG: normal control group; HG: hyperlipidemic +4% DMSO control group; Czzs18: compound C18+4% DMSO; C22: compound C22+4% DMSO; C24: compound C24+4% DMSO; C26: compound C26+4% DMSO; C20: compound C20+4% DMSO; BF: bezafibrate+4% DMSO; TC: total cholesterol; TG: triglyceride; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol. HG is compared to CG.

C18, C22, C24, C26, C20 and BF are compared with HG.

$^{**}p<0.001$, $^{***}p<0.0001$

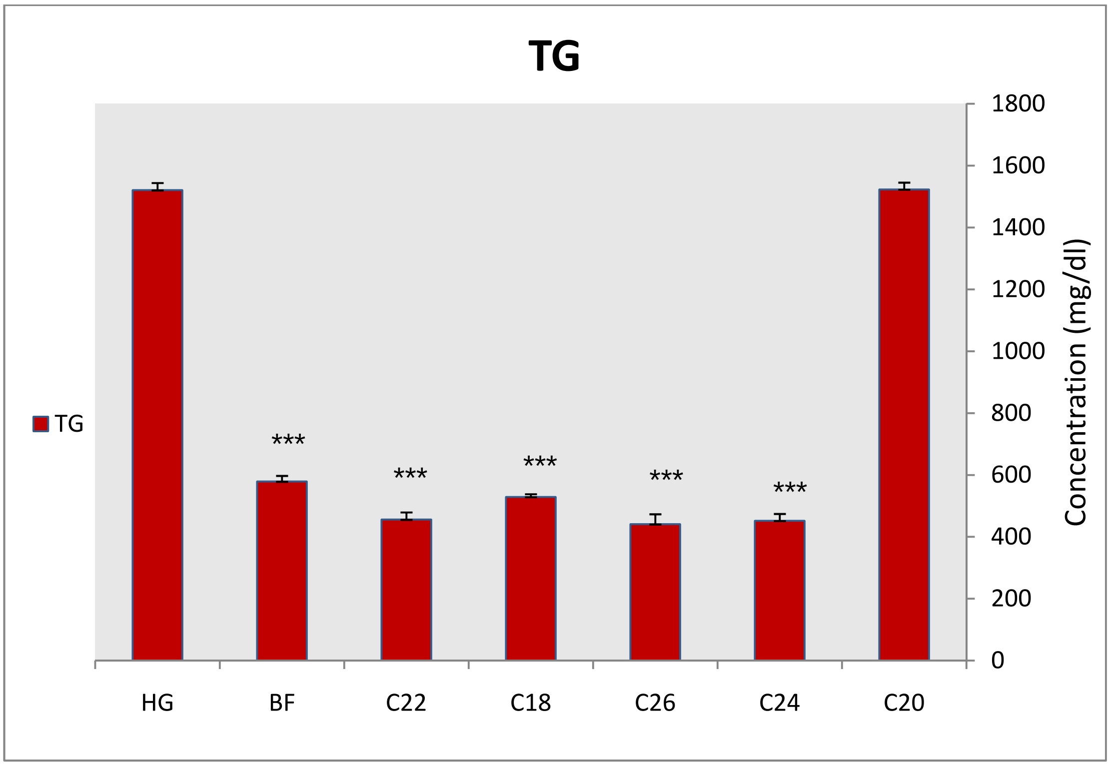


Figure 38. Effect of the novel unsubstituted indole-anthraquinone carboxamide on triglycerides (mg/dl) after 8 hrs of Triton administration.

Values are means ± SEM from six rats in each group. CG: normal control group; HG: hyperlipidemic +4% DMSO control group; C18: compound C18+4% DMSO; C22: compound C22+4% DMSO; C24: compound C24+4% DMSO; C26: compound C26+4% DMSO; C20: compound C20+4% DMSO; BF: bezafibrate+4% DMSO; TC: total cholesterol; TG: triglyceride; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol. HG is compared to CG.

C18, C22, C24, C26, C20 and BF are compared with HG.

$^{***}p<0.0001$

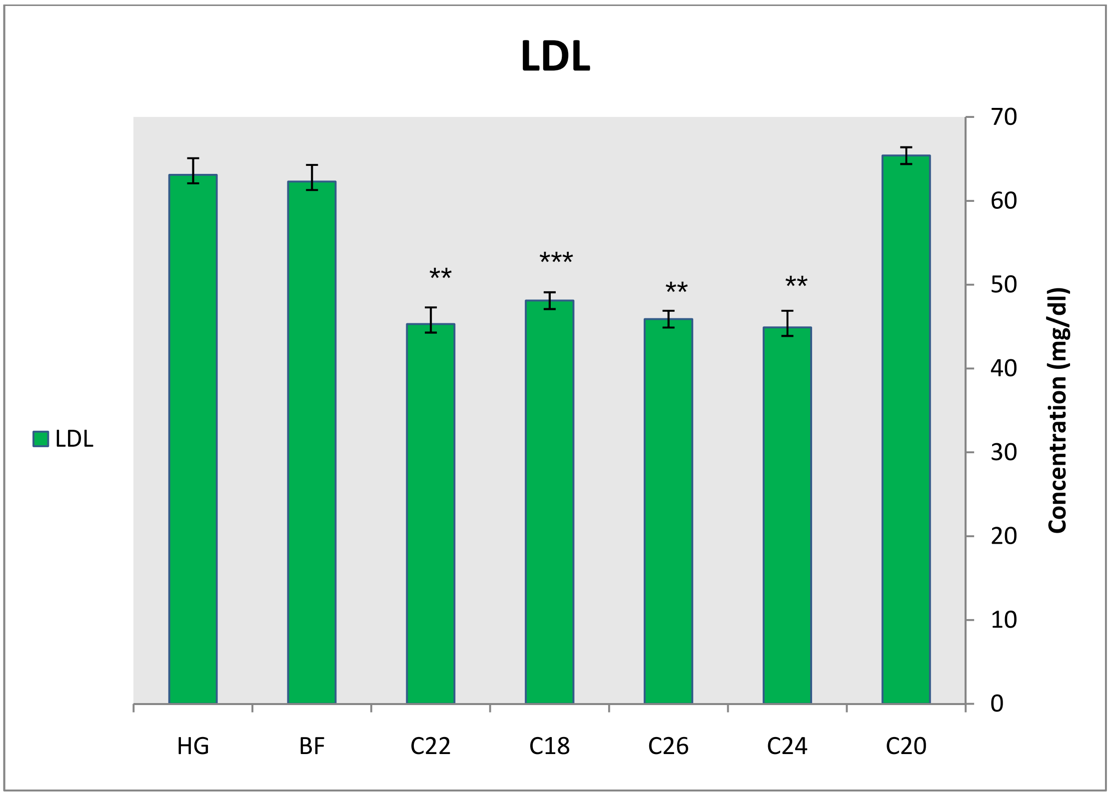


Figure 39. Effect of the novel unsubstituted indole-anthraquinone carboxamide on low-density lipoproteins (mg/dl) after 8 hrs of Triton administration.

Values are means ± SEM from six rats in each group. CG: normal control group; HG: hyperlipidemic +4% DMSO control group; C18: compound C18+4% DMSO; C22: compound C22+4% DMSO; C24: compound C24+4% DMSO; C26: compound C26+4% DMSO; C20: compound C20+4% DMSO; BF: bezafibrate+4% DMSO; TC: total cholesterol; TG: triglyceride; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol. HG is compared to CG.

C18, C22, C24, C26, C20 and BF are compared with HG.

$^{**}p<0.001$, $^{***}p<0.0001$

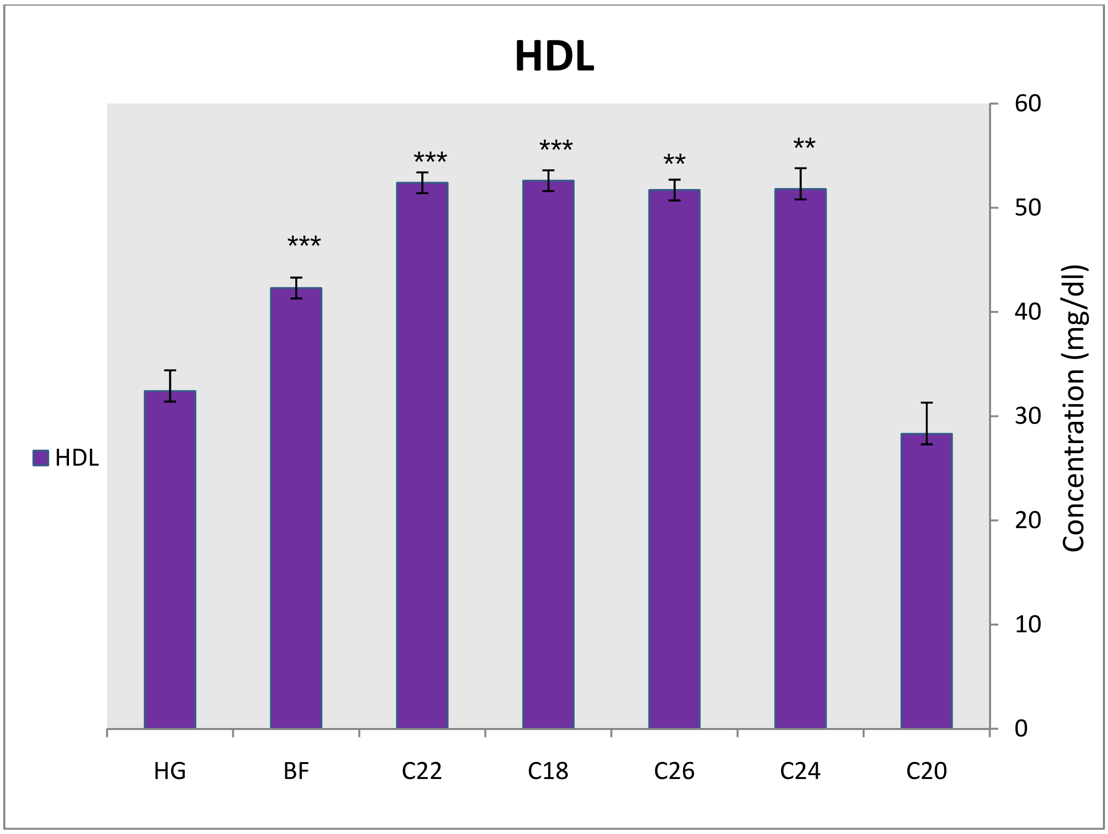


Figure 40. Effect of the novel unsubstituted indole-anthraquinone carboxamide on high-density lipoproteins (mg/dl) after 8 hrs of Triton administration.
Values are means ± SEM from six rats in each group. CG: normal control group; HG: hyperlipidemic +4% DMSO control group; C18: compound C18+4% DMSO; C22: compound C22+4% DMSO; C24: compound C24+4% DMSO; C26: compound C26+4% DMSO; C20: compound C20+4% DMSO; BF: bezafibrate+4% DMSO; TC: total cholesterol; TG: triglyceride; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol. HG is compared to CG.
C18, C22, C24, C26, C20 and BF are compared with HG.
$^{**}p<0.001$, $^{***}p<0.0001$

8.2.5. Effect of novel unsubstituted indole-acetophenone carboxamides group (Series C) and BF on Rat Plasma Lipid Profile:

The plasma TC, TG, HDL-C and LDL-C levels of BF, and unsubstituted indole-acetophenone carboxamide compounds and their derivatives, treated rats 8 hrs after Triton administration are shown in (Table **4**, Fig. **41, 42, 43, 44** and **45**), respectively.

Cholesterol levels:

The results presented in Table **4** indicated that the cholesterol levels decreased (13% $P<0.001$) in compound **C36**, (18% $p< 0.0001$) in compound **C38** with respect to hyperlipidemic control HG. However, no significant difference in plasma total cholesterol levels were observed in compounds **C28, C30, C32** and **BF** compared to HG-treated rats.

Triglyceride levels:

The results presented in Table **4** indicated that the elevated plasma TG levels produced by Triton WR-1339 administration were significantly suppressed (62%, 23% and 56% $p < 0.0001$) in compounds **BF, C36** and **C38** respectively with respect to HG-treated groups. While no significant difference in TG levels were observed in compounds **C28, C30** and **C32** compared to HG-treated rats.

HDL-C levels:

HDL-C levels were significantly increased after 8 h of Triton administration (84%, 74% and 28% $p< 0.0001$) in compounds **C36, C38** and **BF** respectively compared to HG (Table 4). In contrast there was no significant difference in HDL-C levels were observed in compounds **C28, C30** and **C32** compared to HG-treated rats.

LDL levels:

The elevated plasma LDL levels produced by Triton WR-1339 administration were significantly suppressed (15% $p< 0.05$) in compound **C38** only. While no significant

difference in LDL levels were observed in compounds **C28, C30, C32, C36** and **BF** compared to HG-treated rats.

In summary, a significant decrease in TG and TC, with a significant increase in HDL-C plasma levels were observed with compounds **C36** and **C38**. In addition, only compound **C38** showed a reduction in LDL levels.

Table 4. Effect of the novel unsubstituted indole-acetophenone carboxamides and their derivatives on plasma lipid levels in Triton WR-1339-induced hyperlipidemic rats after 8 hrs.

Lipid profile	TC (mg/dl)	TG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
CG	105.5 ± 1.73	126.5 ± 2.99	48.1 ± 0.99	31.3 ± 1.51
HG	223 ± 4.69[c]	1505 ± 23.68[c]	32.3 ± 3.7[c]	62.1 ± 3.05[c]
C28	263 ± 16.90[a]	1534 ± 16.21	27.4 ± 1.97	67.3 ± 1.63
C30	245.8 ± 7.77	1525 ± 13.2	28.8 ± 1.75	64.2 ± 2.7
C32	255 ± 16	1529 ± 7.73	28.5 ± 2.13	66 ± 1.2
C36	196 ± 4.45[b]	1172 ± 16.37[c]	59.7 ± 2.33[c]	66.2 ± 2.58
C38	185 ± 4.37[c]	672 ± 21.35[c]	56.5 ± 1.64[c]	53.4 ± 1.24[a]
BF	216.8 ± 6.18	566.5 ± 15.6[c]	43.7 ± 1.56[c]	63.2 ± 1.98

Values are means ± SEM from six rats in each group. CG: normal control group; HG: hyperlipidemic +4% DMSO control group; C28: compound C28+4% DMSO; C30: compound C30+4% DMSO; C32: compound C32+4% DMSO; C36: compound C36+4% DMSO; C38: compound C38+4% DMSO; BF: bezafibrate+4% DMSO; TC: total cholesterol; TG: triglyceride; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol. HG is compared to CG.

C28, C30, C32, C36, C38 and BF are compared with HG.

[a] $p<0.01$, [b] $p<0.001$, [c] $p<0.0001$

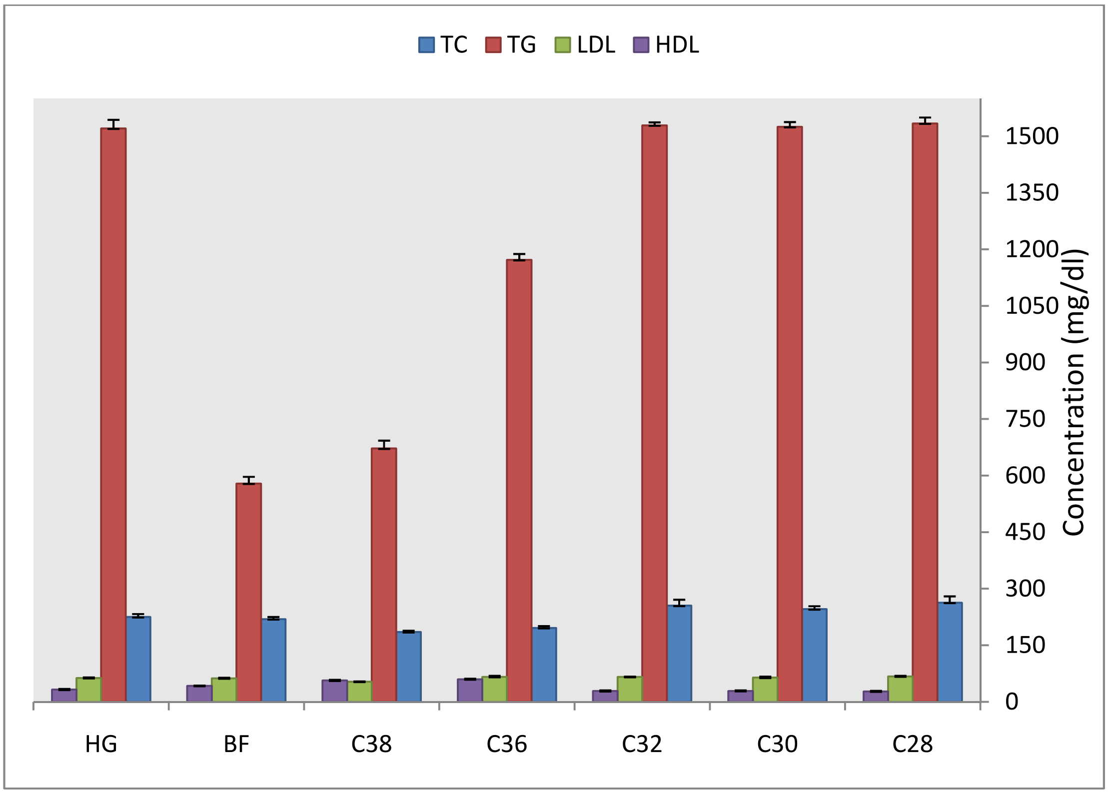


Figure 41. Effect of the novel unsubstituted indole-acetophenone carboxamides and their derivatives on lipid profile (mg/dl) after 8 hrs of Triton administration.

Values are means ± SEM from six rats in each group. CG: normal control group; HG: hyperlipidemic +4% DMSO control group; C28: compound C28+4% DMSO; C30: compound C30+4% DMSO; C32: compound C32+4% DMSO; C36: compound C36+4% DMSO; C38: compound C38+4% DMSO; BF: bezafibrate+4% DMSO; TC: total cholesterol; TG: triglyceride; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol. HG is compared to CG.

C28, C30, C32, C36, C38 and BF are compared with HG.

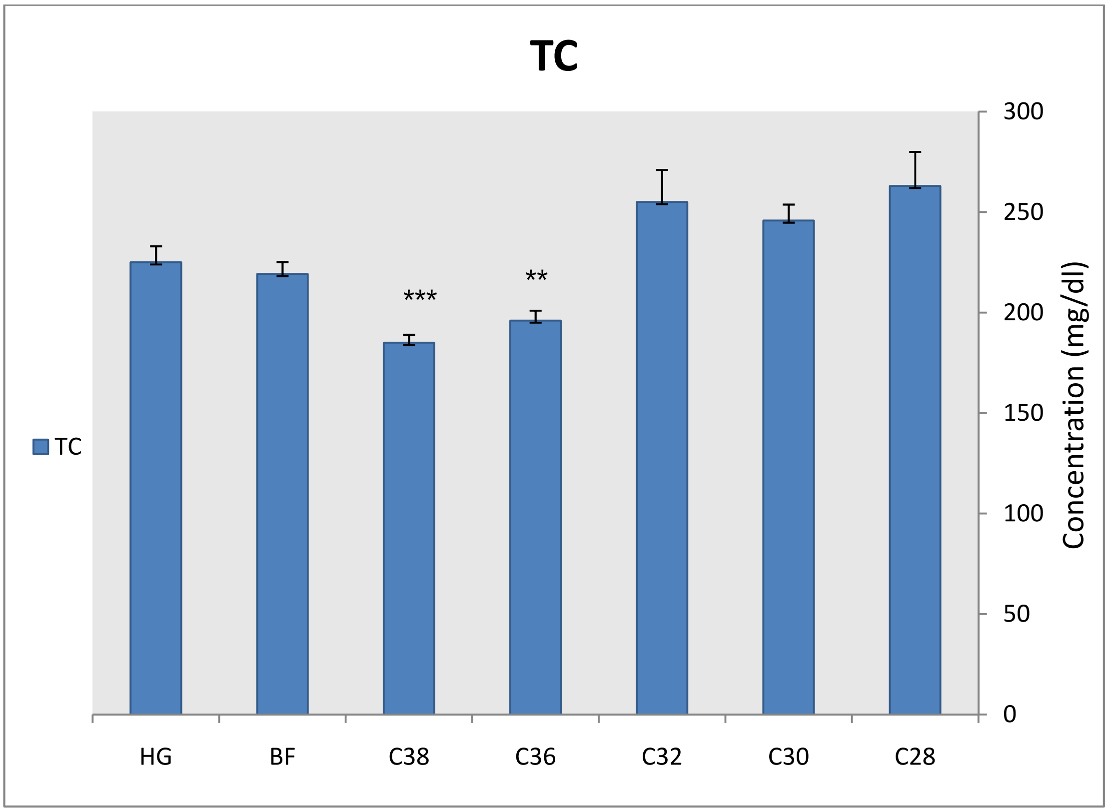


Figure 42. Effect of the novel unsubstituted indole-acetophenone carboxamides and their derivatives on total cholestrol (mg/dl) after 8 hrs of Triton administration.
Values are means ± SEM from six rats in each group. CG: normal control group; HG: hyperlipidemic +4% DMSO control group; C28: compound C28+4% DMSO; C30: compound C30+4% DMSO; C32: compound C32+4% DMSO; C36: compound C36+4% DMSO; C38: compound C38+4% DMSO; BF: bezafibrate+4% DMSO; TC: total cholesterol; TG: triglyceride; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol. HG is compared to CG.
C28, C30, C32, C36, C38 and BF are compared with HG.

** $p<0.001$, *** $p<0.0001$

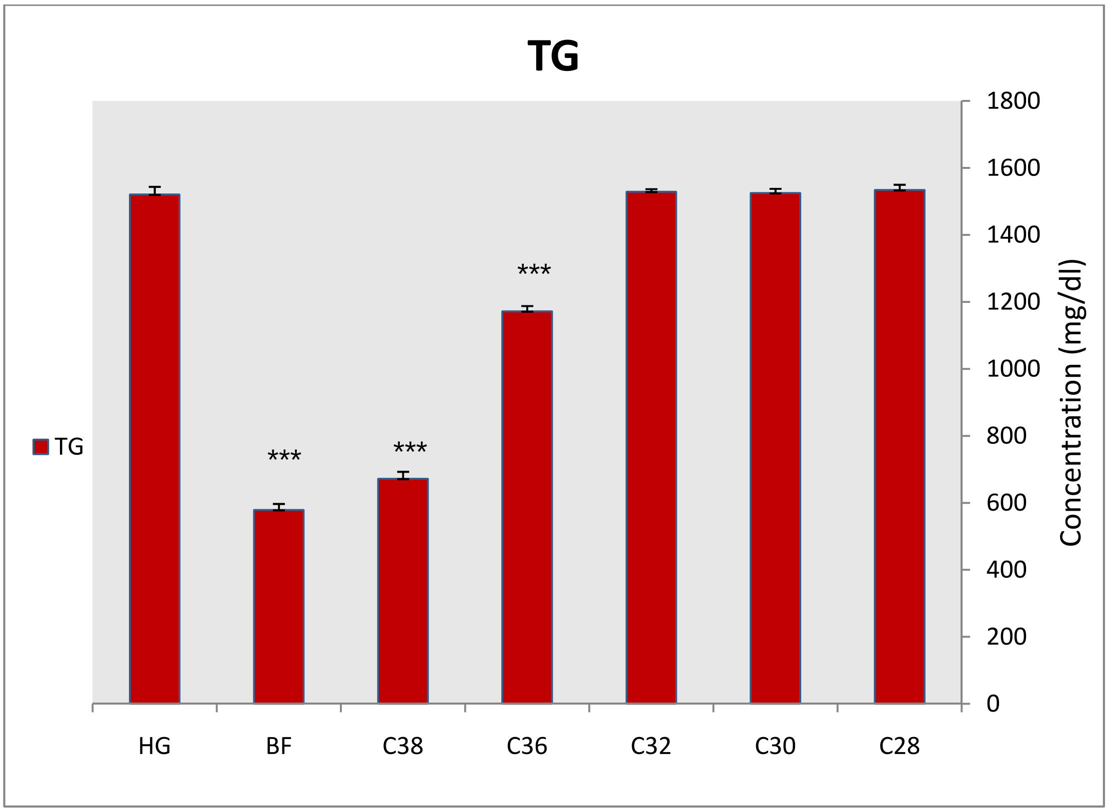


Figure 43. Effect of the novel unsubstituted indole-acetophenone carboxamides and their derivatives on triglycerides (mg/dl) after 8 hrs of Triton administration.
Values are means ± SEM from six rats in each group. CG: normal control group; HG: hyperlipidemic +4% DMSO control group; C28: compound C28+4% DMSO; C30: compound C30+4% DMSO; C32: compound C32+4% DMSO; C36: DMSO; TC: total cholesterol; TG: triglyceride; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol. HG is compared to CG.
C28, C30, C32, C36, C38 and BF are compared with HG.

*** $p<0.0001$

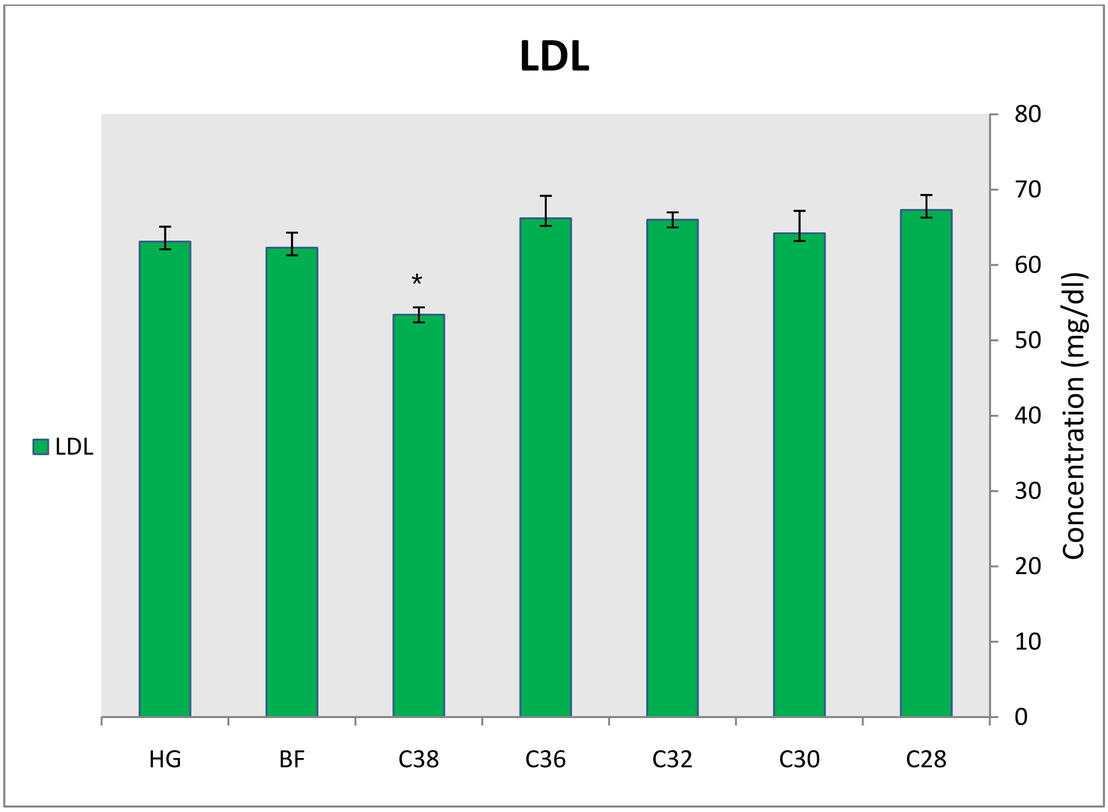


Figure 44. Effect of the novel unsubstituted indole-acetophenone carboxamides and their derivatives on low-density lipoproteins (mg/dl) after 8 hrs of Triton administration. Values are means ± SEM from six rats in each group. CG: normal control group; HG: hyperlipidemic +4% DMSO control group; C28: compound C28+4% DMSO; C30: compound C30+4% DMSO; C32: compound C32+4% DMSO; C36: compound C36+4% DMSO; C38: compound C38+4% DMSO; BF: bezafibrate+4% DMSO; TC: total cholesterol; TG: triglyceride; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol. HG is compared to CG.
C28, C30, C32, C36, C38 and BF are compared with HG.

* $p<0.01$

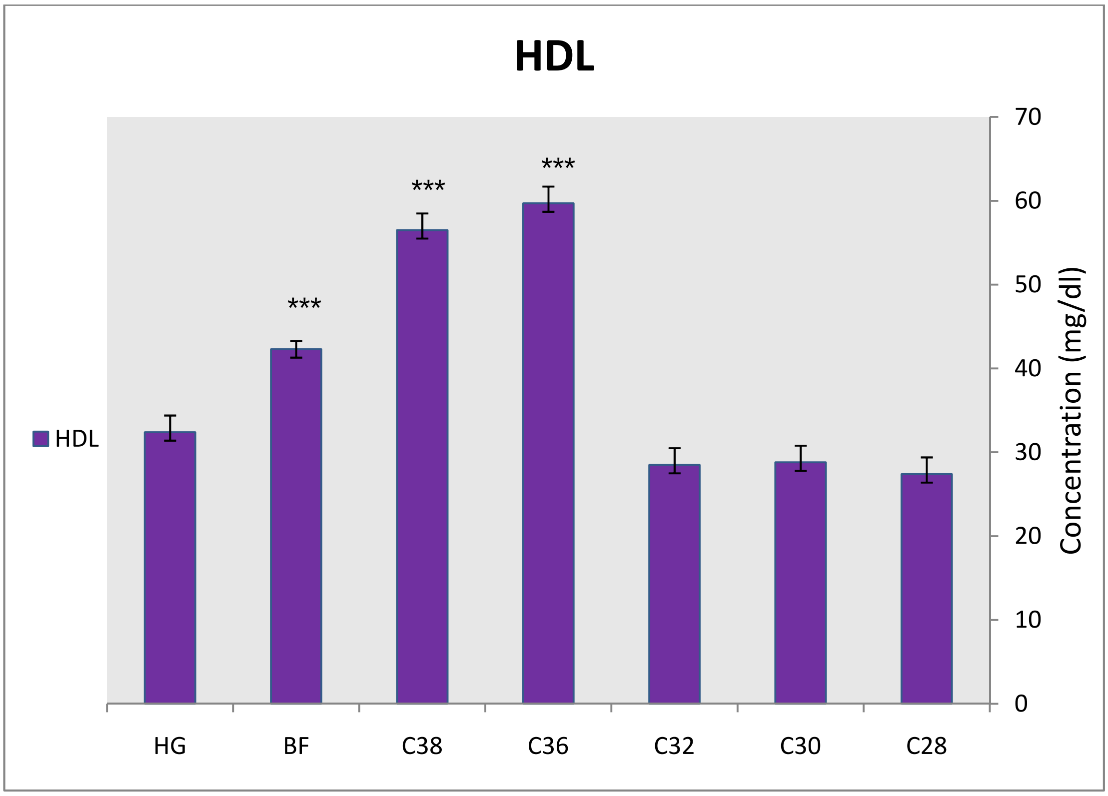


Figure 45. Effect of the novel unsubstituted indole-acetophenone carboxamides and their derivatives on high-density lipoprotein (mg/dl) after 8 hrs of Triton administration. Values are means ± SEM from six rats in each group. CG: normal control group; HG: hyperlipidemic +4% DMSO control group; C28: compound C28+4% DMSO; C30: compound C30+4% DMSO; C32: compound C32+4% DMSO; C36: compound C36+4% DMSO; C38: compound C38+4% DMSO; BF: bezafibrate+4% DMSO; TC: total cholesterol; TG: triglyceride; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol. HG is compared to CG.
C28, C30, C32, C36, C38 and BF are compared with HG.

***$p<0.0001$

8.2.6. Effect of novel unsubstituted and substituted indole benzene carboxamides group (Series D) and BF on Rat Plasma Lipid Profile:

The plasma TC, TG, HDL-C and LDL-C levels of BF and unsubstituted indole benzene and substituted benzene carboxamide compounds, treated rats 8 hrs after Triton administration are shown in (Table **5** and Fig. **46**), respectively.

The results presented in Table 5 indicated that there were no significant difference in plasma TC, TG, HDL-C and LDL-C levels in the synthesized compounds compared to HG-treated rats.

Table 5. Effect of the novel unsubstituted and substituted benzene indole carboxamides and their derivatives on plasma lipid levels in Triton WR-1339-induced hyperlipidemic rats after 8 hrs.

Lipid profile	TC (mg/dl)	TG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
CG	106.6 ± 2.11	127.8 ± 276	49.8 ± 0.71	33.4 ± 0.98
HG	218 ± 5.77^{c}	1520 ± 18.25^{c}	33.7 ± 1.9^{c}	63.5 ± 2.32^{c}
C47	258 ± 17.92	1530 ± 19.39	28.9 ± 2.32	66.1 ± 1.64
C57	255 ± 13.64	1529 ± 21.60	28.7 ± 2.02	66.8 ± 1.67
C63	258 ± 16.98	1530 ± 19.02	28.3 ± 2.23	64.3 ± 2.04
C51	272 ± 21.72	1526 ± 21.51	26.2 ± 1.94	65.8 ± 1.73
C53	276 ± 32.09	1577 ± 36.25	30.0 ± 1.95	66.6 ± 1.36
BF	216.4± 5.22	563 ± 19.7^{c}	44.4 ± 0.98^{c}	61.7 ± 1.95

Values are means ± SEM from six rats in each group. CG: normal control group; HG: hyperlipidemic +4% DMSO control group; C47: compound C47+4% DMSO; C57: compound C57 +4% DMSO; C63: compound C63+4% DMSO; C51: compound C51+4% DMSO; C53: compound C53 +4% DMSO; BF: bezafibrate+4% DMSO; TC: total cholesterol; TG: triglyceride; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol. HG is compared to CG.
C47, C57, C63, C51, C53 and BF are compared with HG.
^{c} $p<0.0001$

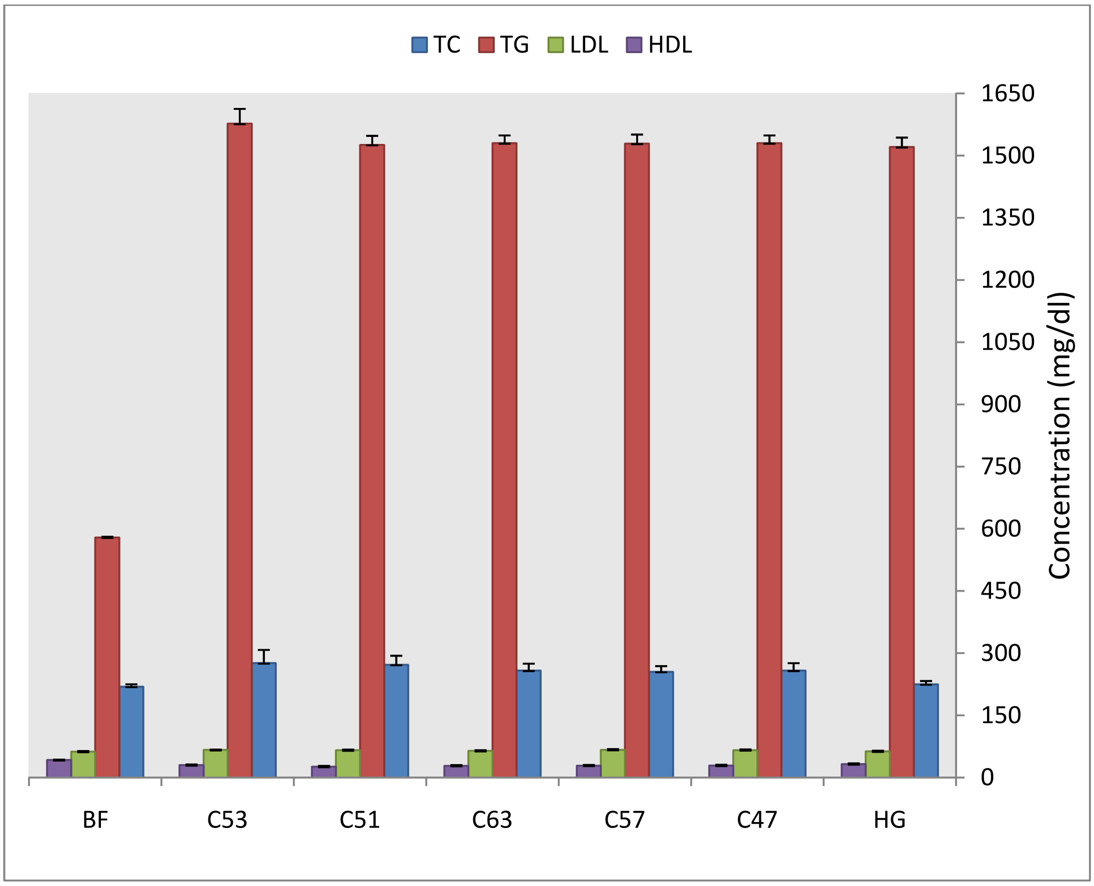


Figure 46. Effect of the novel unsubstituted and substituted benzene indole carboxamides and their derivatives on lipid profile (mg/dl) after 8 hrs of Triton administration.

Values are means ± SEM from six rats in each group. CG: normal control group; HG: hyperlipidemic +4% DMSO control group; C47: compound C47+4% DMSO; C57: compound C57 +4% DMSO; C63: compound C63+4% DMSO; C51: compound C51+4% DMSO; C53: compound C53 +4% DMSO; BF: bezafibrate+4% DMSO; TC: total cholesterol; TG: triglyceride; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol. HG is compared to CG.

C47, C57, C63, C51, C53 and BF are compared with HG.

8.2.7. Effect of novel unsubstituted indole benzyl and phenyl-ethyl carboxamides group (Series E) and BF on Rat Plasma Lipid Profile:

The plasma TC, TG, HDL-C and LDL-C levels of BF and unsubstituted indole benzyl and phenylethyl carboxamide compounds, treated rats 8 hrs after Triton administration are shown in (Table **6** and Fig. **47**), respectively.

The results presented in Table 6 indicated that there were no significant difference in plasma TC, TG, HDL-C and LDL-C levels in the synthesized compounds compared to HG-treated rats.

Table 6. Effect of the novel unsubstituted indole-benzyl and phenyl ethyl carboxamides and their derivatives on plasma lipid levels in Triton WR-1339-induced hyperlipidemic rats after 8 hrs.

Lipid profile	TC (mg/dl)	TG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
CG	110 ± 1.92	129.1 ± 2..87	47.9 ± 1.13	31..8 ± 1.40
HG	211.2 ± 5.87^{c}	1522.4 ± 22.5	31.7 ± 2.5^{c}	62.7 ± 2.76^{c}
C73	269 ± 16.62	1538 ± 17.47	27.4 ± 2.65	64.6 ± 1.76
C75	267 ± 15.06	1541 ± 22.62	27.1 ± 1.85	68.2 ± 3.09
C67	260 ± 13.88	1539 ± 20.37	28.2 ± 1.74	67.2 ± 1.81
BF	218.8 ± 5.14	568 ± 19.3^{c}	44.7 ± 1.29^{c}	63.6 ± 1. 97

Values are means ± SEM from six rats in each group. CG: normal control group; HG: hyperlipidemic +4% DMSO control group; C73: compound C73 +4% DMSO; C75: compound C75 +4% DMSO; C67: compound C67 +4% DMSO; BF: bezafibrate+4% DMSO; TC: total cholesterol; TG: triglyceride; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol. HG is compared to CG.
C73, C75, C67and BF are compared with HG.
^{c} $p<0.0001$

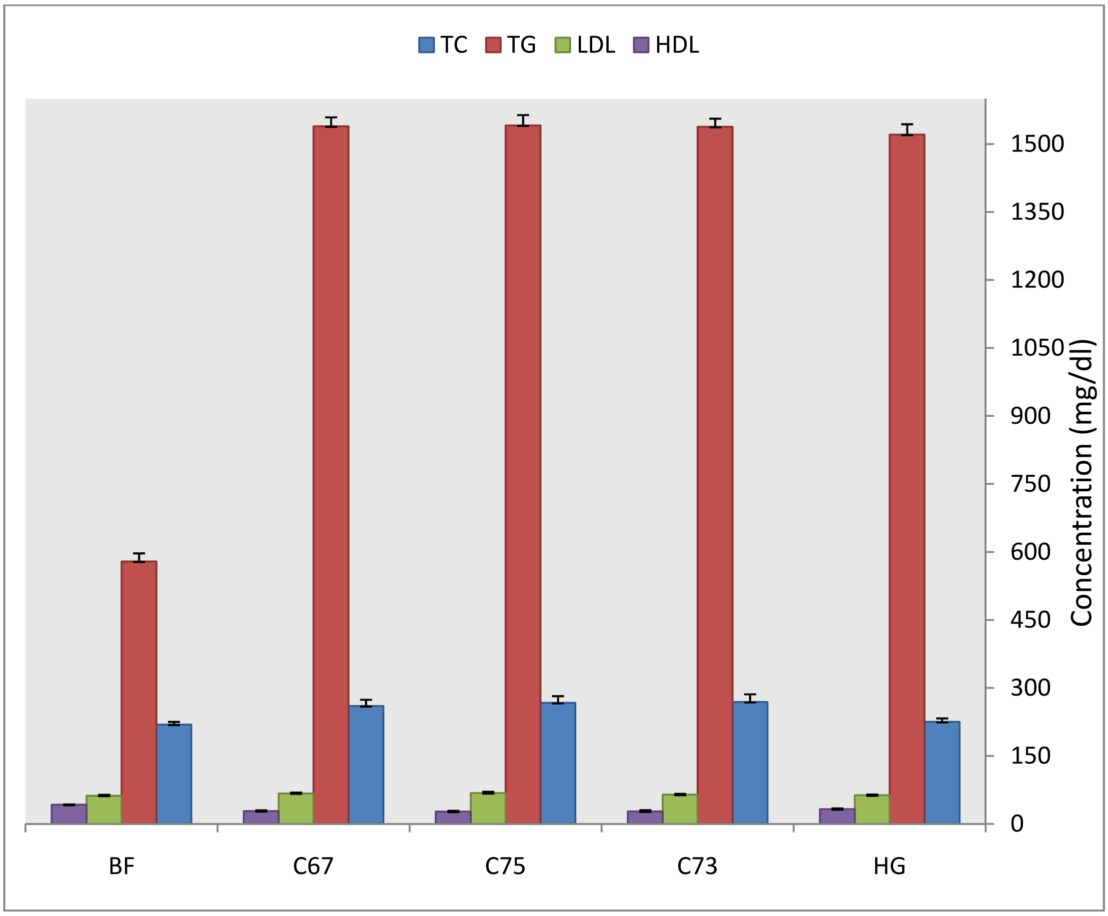


Figure 47. Effect of the novel unsubstituted indole-benzyl and phenyl ethyl carboxamides and their derivatives on lipid profile (mg/dl) after 8 hrs of Triton administration.

Values are means ± SEM from six rats in each group. CG: normal control group; HG: hyperlipidemic +4% DMSO control group; C73: compound C73 +4% DMSO; C75: compound C75 +4% DMSO; C67: compound C67 +4% DMSO; BF: bezafibrate+4% DMSO; TC: total cholesterol; TG: triglyceride; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol. HG is compared to CG.
C73, C75, C67and BF are compared with HG.

8.2.8. Effect of unsubstituted indole heterocyclic carboxamides group (Series F) and BF on Rat Plasma Lipid Profile:

The plasma TC, TG, HDL-C and LDL-C levels of BF and the miscellaneous unsubstituted indole carboxamide compounds, treated rats 8 hrs after Triton administration are shown in (Table **7** and Fig. **48**), respectively.

The results presented in Table 7 indicated that there were no significant difference in plasma TC, TG, HDL-C and LDL-C levels in the synthesized compounds compared to HG-treated rats.

Table 7. Effect of unsubstituted indole heterocyclic carboxamides derivatives on plasma lipid levels in Triton WR-1339-induced hyperlipidemic rats after 8 hrs.

Lipid profile	TC (mg/dl)	TG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
CG	107 ± 1.84	128.5 ± 3.95	49.4 ± 0.89	30.4 ± 1.12
HG	225 ± 7.78[c]	1520.8 ± 22.86[c]	32.4 ± 2.0[c]	63.1 ± 2.15[c]
C85	265 ± 26.45	1534 ± 15.43	27.8 ± 2.15	66.0 ± 1.58
C83	278 ± 16.98[a]	1521 ± 17.21	31.5 ± 1.62	62.5 ± 1.93
C79	267 ± 21.72	1526 ± 19.4	28.9 ± 1.44	63.9 ± 1.53
BF	219.2 ± 6.18	579 ± 18.4[c]	42.3 ± 1.24[c]	62.3 ± 2.08

Values are means ± SEM from six rats in each group. CG: normal control group; HG: hyperlipidemic +4% DMSO control group; C85: compound C85+4% DMSO; C83: compound C83+4% DMSO; C79: compound C79+4% DMSO; BF: bezafibrate+4% DMSO; TC: total cholesterol; TG: triglyceride; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol. HG is compared to CG.

C85, C83, C79 and BF are compared with HG.

[a] $p<0.05$, [c] $p<0.0001$

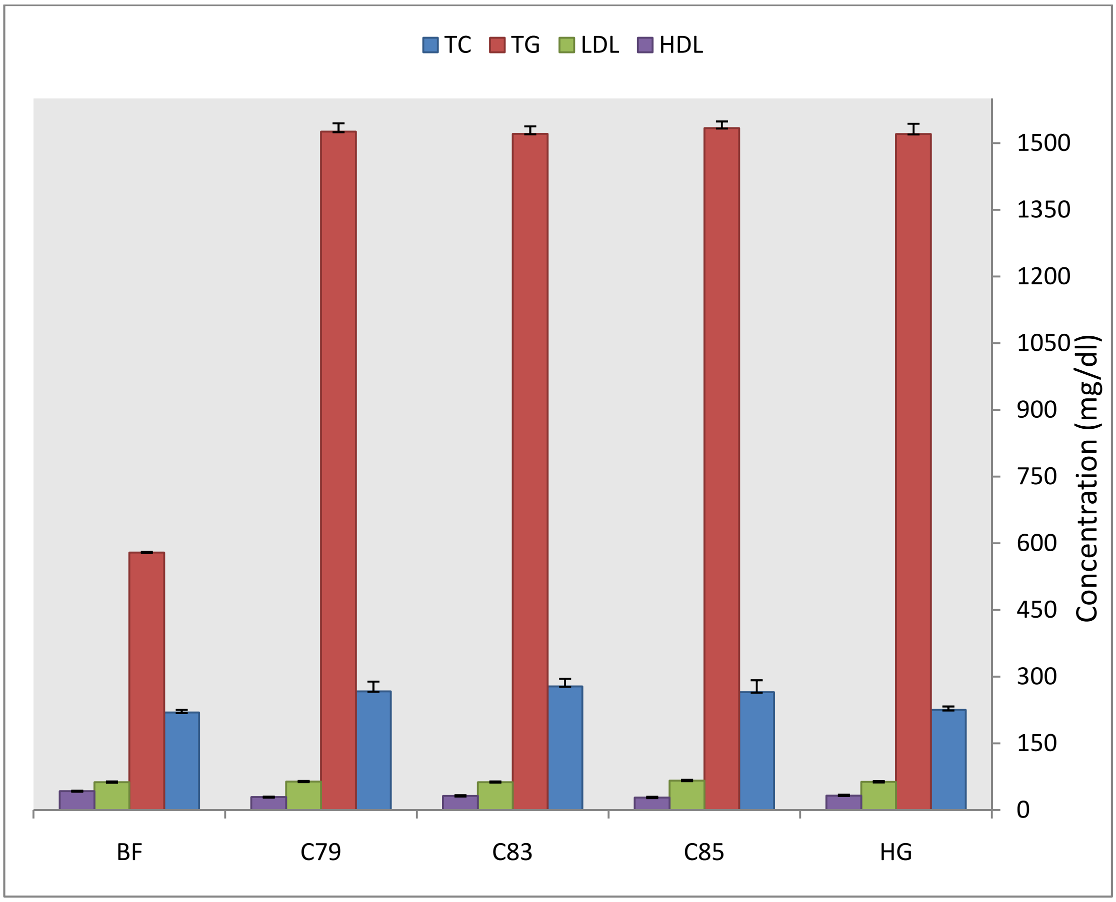


Figure 48. Effect of unsubstituted indole heterocyclic carboxamides derivatives on lipid profile (mg/dl) after 8 hrs of Triton administration.

Values are means ± SEM from six rats in each group. CG: normal control group; HG: hyperlipidemic +4% DMSO control group; C85: compound C85+4% DMSO; C83: compound C83+4% DMSO; C79: compound C79+4% DMSO; BF: bezafibrate+4% DMSO; TC: total cholesterol; TG: triglyceride; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol. HG is compared to CG.
C85, C83, C79 and BF are compared with HG.

8.2.9. Effect of novel substituted indole carboxamides group (Series G) and BF on Rat Plasma Lipid Profile:

The plasma TC, TG, HDL-C and LDL-C levels of BF, and benzophenone compounds treated rats 8 hrs after Triton administration are shown in (Table **8**, Fig. **49, 50, 51, 52** and **53**), respectively.

Cholesterol levels:

The results presented in Table 8 indicated that the elevated plasma TC levels produced by Triton WR-1339 administration were significantly suppressed (21% and 20% $P<0.0001$) in compounds **C88** and **C90** respectively with respect to hyperlipidemic control HG. However, no significant differences in plasma total cholesterol levels between any treated groups (**BF**, **C93, C96** and **C97**) were observed in comparison to HG-treated rats.

Triglyceride levels:

The results presented in Table 8 indicated that the elevated plasma TG levels produced by Triton WR-1339 administration were significantly suppressed (62%, 71% and 70% $p< 0.0001$) in compounds **BF, C93, C88** and **C90** respectively with respect to hyperlipidemic control HG. While no significant difference in TG levels were observed in compounds **C96** and **C97** compared to HG-treated rats.

HDL-C levels:

HDL-C levels were significantly increased after 8 h of Triton administration (61%, 56% and 28% $p<0.001$) in compounds **C93, C88, C90** and **BF** respectively compared to HG (Table 8). In contrast no significant difference in HDL-C levels were observed in compounds **C96** and **C97** compared to HG-treated rats.

LDL levels:

The elevated plasma LDL levels produced by Triton WR-1339 administration were significantly suppressed (29% and 31% $p < 0.0001$) in **C93, C88** and **C90** respectively compared to HG. While no significant difference in LDL levels were observed in **C96, C97** and **BF** compared to HG-treated rats.

In summary, a significant decrease in TG and LDL and an increase in HDL-C plasma levels were observed with compounds **C93, C88** and **C90**. In addition, compounds **C88** and **C90** showed a significant reduction in TC levels too.

Table 8. Effect of novel substituted indole carboxamides on plasma lipid levels in Triton WR-1339-induced hyperlipidemic rats after 8 hrs.

Lipid profile	TC (mg/dl)	TG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
CG	107 ± 1.84	128.5 ± 3.95	49.4 ± 0.89	30.4 ± 1.12
HG	225 ± 7.78^{c}	1520.8 ± 22.86^{c}	32.4 ± 2.0^{c}	63.1 ± 2.15^{c}
C88	176 ± 6.21^{c}	442 ± 21.02^{c}	52.2 ± 1.74^{b}	45 ± 1.96^{c}
C93	187 ± 5.5^{c}	433.8 ± 19.9^{c}	53.2 ± 1.55^{b}	44 ± 1.65^{c}
C90	179 ± 4.41^{c}	454 ± 37.35^{c}	50.6 ± 2.09^{b}	43.6 ± 1.23^{c}
C96	246 ± 18.25	1533 ± 11.35	28.8 ± 1.88	66.5 ± 1.23
C97	278 ± 29.97	1585 ± 50.31	29.1 ± 1.35	64.3 ± 1.62
BF	219.2 ± 6.18	579 ± 18.4^{c}	42.3 ± 1.24^{c}	62.3 ± 2.08

Values are means ± SEM from six rats in each group. CG: normal control group; HG: hyperlipidemic +4% DMSO control group; C88: compound C88+4% DMSO; C93: compound C93+4% DMSO; C90: compound C90+4% DMSO; C96: compound C96 +4% DMSO; C97: compound C97+4% DMSO; BF: bezafibrate+4% DMSO; TC: total cholesterol; TG: triglyceride; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol. HG is compared to CG.
C88, C93, C90, C96, C97and BF are compared with HG.

^{b} $p<0.001$, ^{c} $p<0.0001$

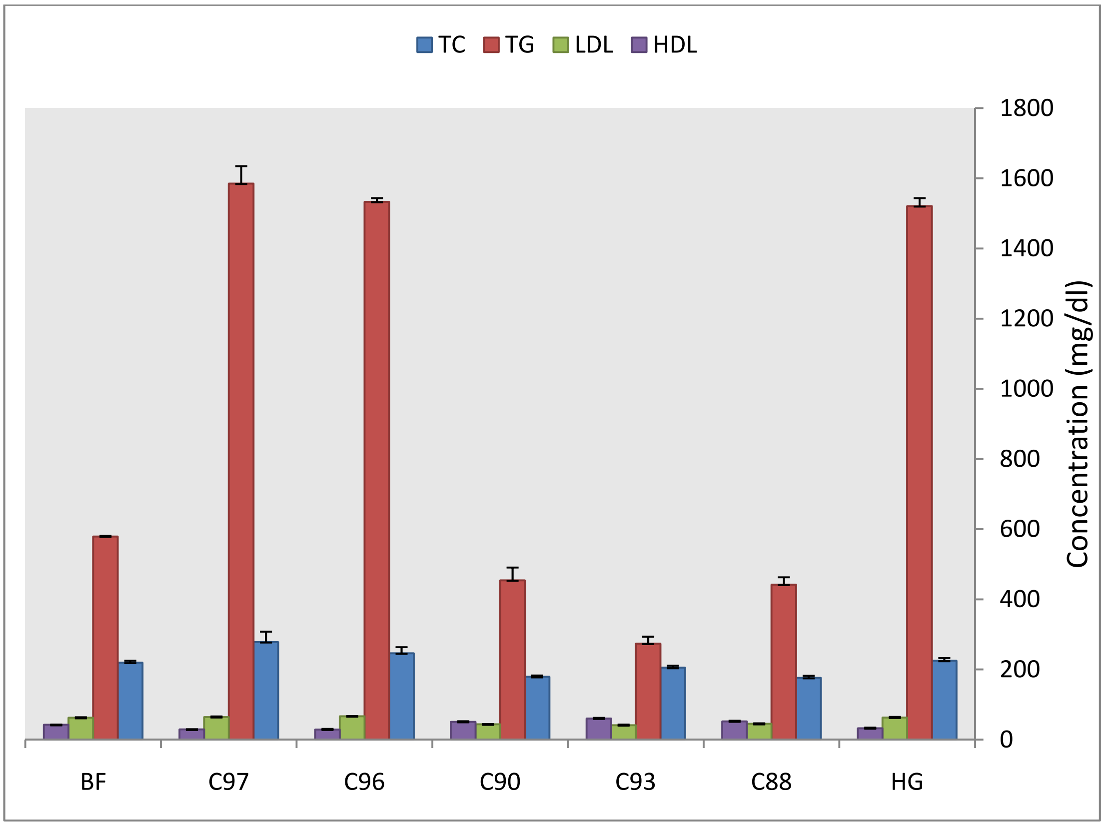


Figure 49. Effect of novel substituted indole carboxamides on lipid profile (mg/dl) after 8 hrs of Triton administration.

Values are means ± SEM from six rats in each group. CG: normal control group; HG: hyperlipidemic +4% DMSO control group; C88: compound C88+4% DMSO; C93: compound C93+4% DMSO; C90: compound C90+4% DMSO; C96: compound C96 +4% DMSO; C97: compound C97+4% DMSO; BF: bezafibrate+4% DMSO; TC: total cholesterol; TG: triglyceride; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol. HG is compared to CG.

C88, C93, C90, C96, C97and BF are compared with HG.

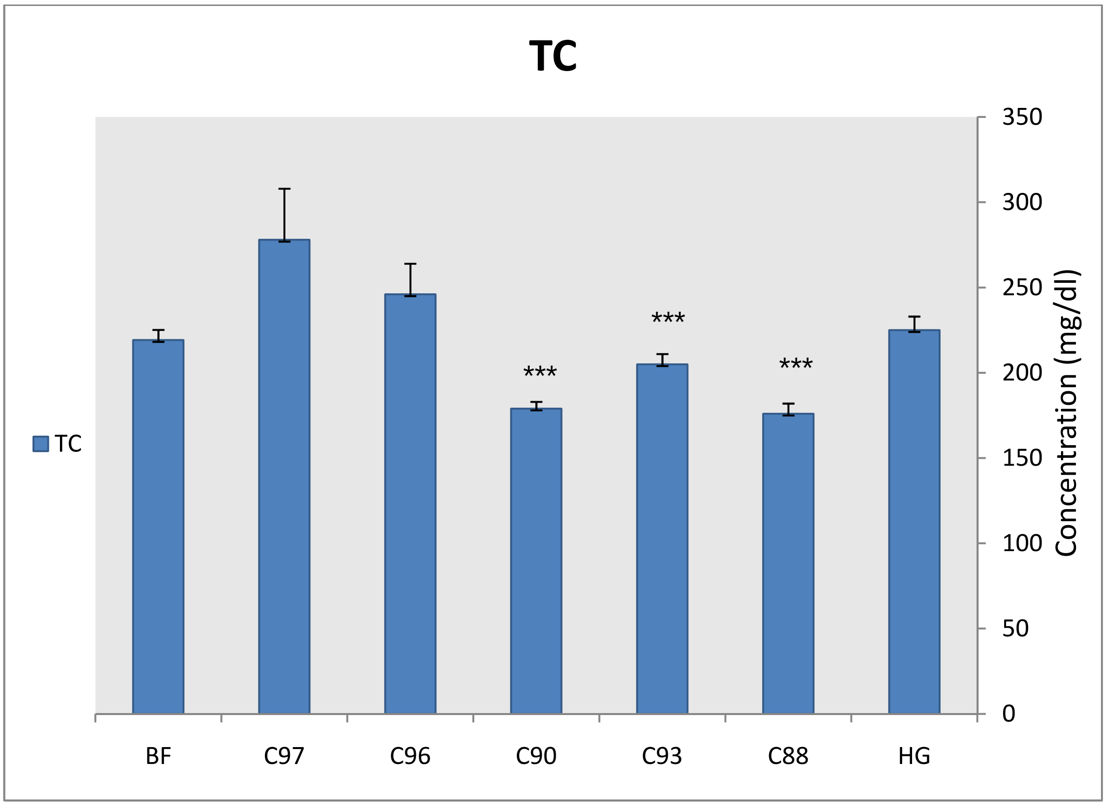


Figure 50. Effect of novel substituted indole carboxamides on total cholestrol (mg/dl) after 8 hrs of Triton administration.

Values are means ± SEM from six rats in each group. CG: normal control group; HG: hyperlipidemic +4% DMSO control group; C88: compound C88+4% DMSO; C93: compound C93+4% DMSO; C90: compound C90+4% DMSO; C96: compound C96 +4% DMSO; C97: compound C97+4% DMSO; BF: bezafibrate+4% DMSO; TC: total cholesterol; TG: triglyceride; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol. HG is compared to CG.

C88, C93, C90, C96, C97and BF are compared with HG.

*** $p<0.0001$

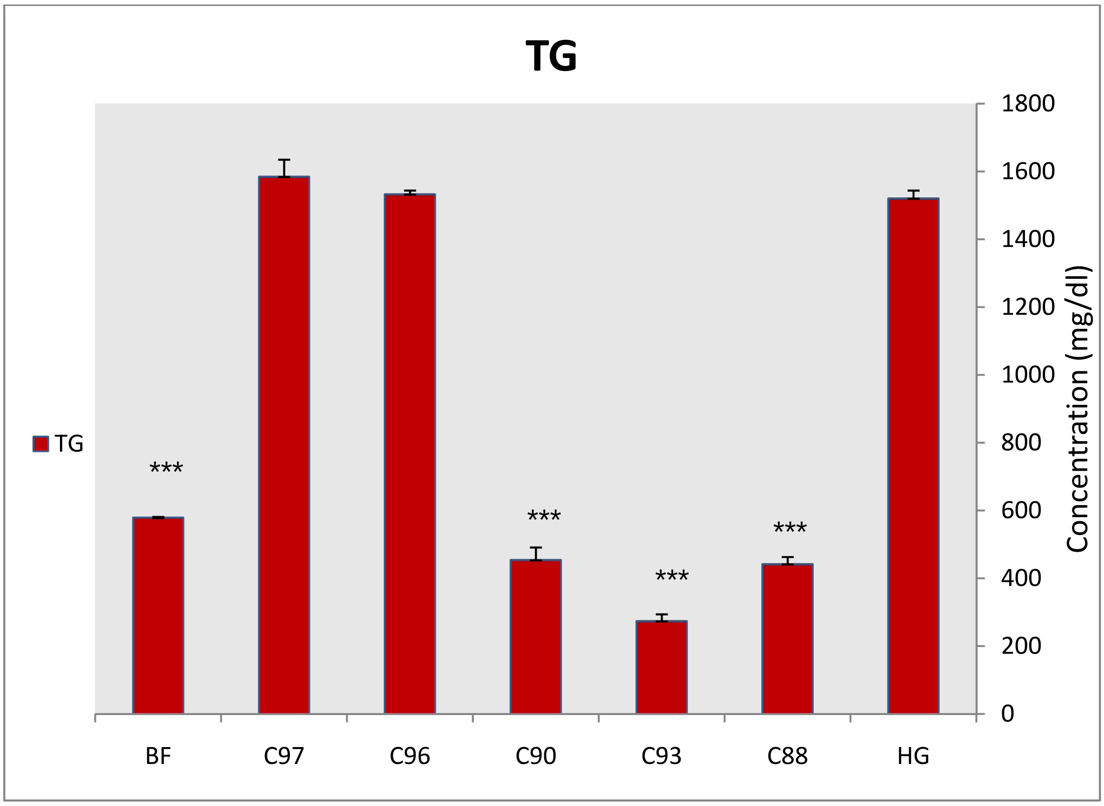


Figure 51. Effect of novel substituted indole carboxamides on triglycerides (mg/dl) after 8 hrs of Triton administration.

Values are means ± SEM from six rats in each group. CG: normal control group; HG: hyperlipidemic +4% DMSO control group; C88: compound C88+4% DMSO; C93: compound C93+4% DMSO; C90: compound C90+4% DMSO; C96: compound C96 +4% DMSO; C97: compound C97+4% DMSO; BF: bezafibrate+4% DMSO; TC: total cholesterol; TG: triglyceride; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol. HG is compared to CG.

C88, C93, C90, C96, C97and BF are compared with HG.

*** $p<0.0001$

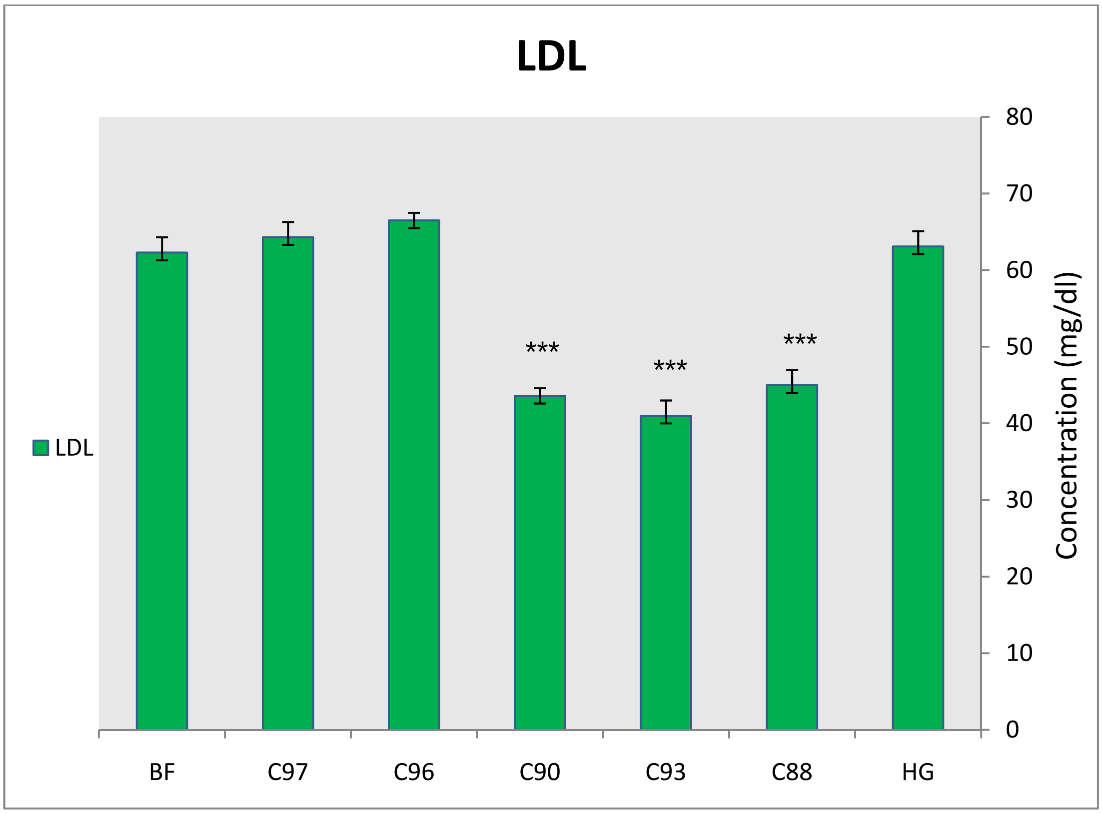


Figure 52. Effect of novel substituted indole carboxamides on low density lipoproteins (mg/dl) after 8 hrs of Triton administration.
Values are means ± SEM from six rats in each group. CG: normal control group; HG: hyperlipidemic +4% DMSO control group; C88: compound C88+4% DMSO; C93: compound C93+4% DMSO; C90: compound C90+4% DMSO; C96: compound C96 +4% DMSO; C97: compound C97+4% DMSO; BF: bezafibrate+4% DMSO; TC: total cholesterol; TG: triglyceride; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol. HG is compared to CG.
C88, C93, C90, C96, C97and BF are compared with HG.

** p<0.001

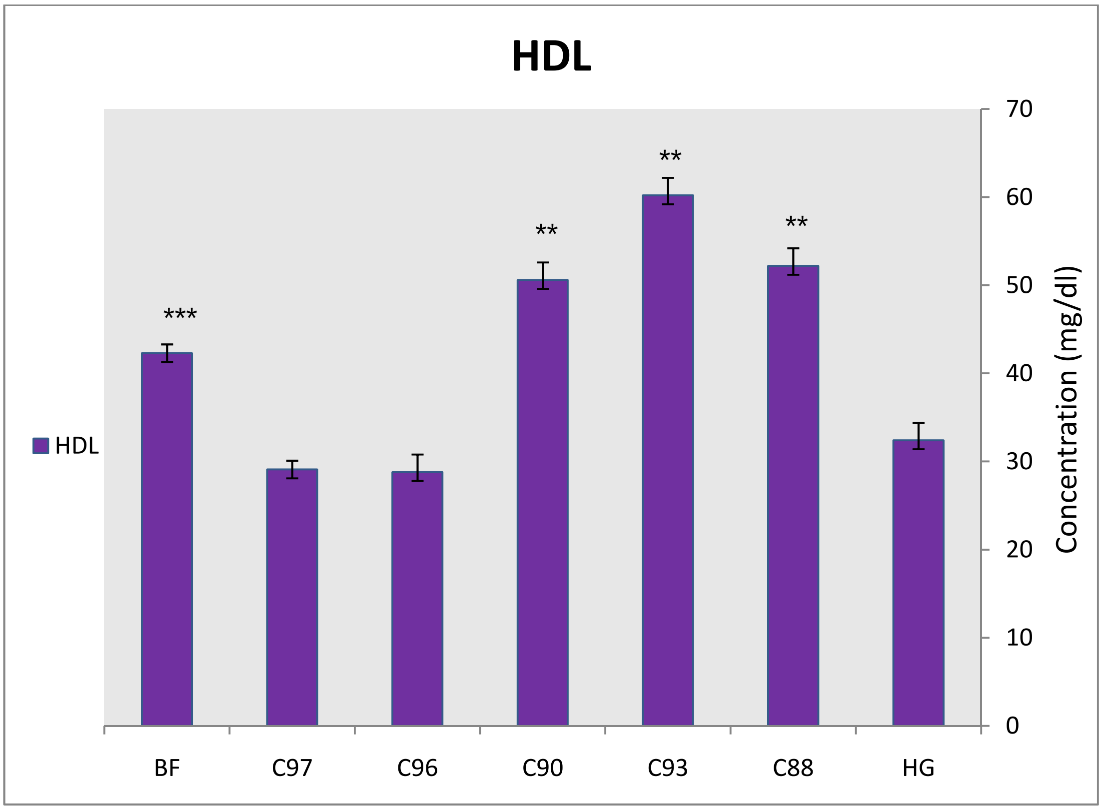


Figure 53. Effect of novel substituted indole carboxamides on high-density lipoproteins (mg/dl) after 8 hrs of Triton administration.

Values are means ± SEM from six rats in each group. CG: normal control group; HG: hyperlipidemic +4% DMSO control group; C88: compound C88+4% DMSO; C93: compound C93+4% DMSO; C90: compound C90+4% DMSO; C96: compound C96 +4% DMSO; C97: compound C97+4% DMSO; BF: bezafibrate+4% DMSO; TC: total cholesterol; TG: triglyceride; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol. HG is compared to CG.

C88, C93, C90, C96, C97and BF are compared with HG.

*** $p<0.0001$, ** $p<0.001$

8.2.10. Effect of removal of benzene group from indole carboxamides group (Series H) and BF on Rat Plasma Lipid Profile:

The plasma TC, TG, HDL-C and LDL-C levels of BF, and benzophenone compounds treated rats 8 hrs after Triton administration are shown in (Table **9**, Fig. **54, 55, 56, 57** and **58**), respectively.

Cholesterol levels:

The results presented in Table 9 indicated that the elevated plasma TC levels produced by Triton WR-1339 administration were significantly suppressed (24%, 23% and 20% P<0.0001) in compounds **C103, C104** and **C111** respectively with respect to hyperlipidemic control HG. However, no significant differences in plasma total cholesterol levels between any treated groups (**BF**, compounds **C101, C110** and **C108**) were observed in comparison to HG-treated rats.

Triglyceride levels:

The results presented in Table 9 indicated that the elevated plasma TG levels produced by Triton WR-1339 administration were significantly suppressed (62%, 67%, 71%, 69% and 68% p< 0.0001) in compounds **BF, C110, C103, C104** and **C111** respectively with respect to hyperlipidemic control HG. While no significant difference in TG levels were observed in compounds **C101** and **C108** compared to HG-treated rats.

HDL-C levels:

HDL-C levels were significantly increased after 8 h of Triton administration (64% p<0.0001) in compound **C110** (56%, 56%, 60% and 28% p<0.001) in compounds **C103, C104, C111** and **BF** respectively compared to HG (Table 9). In contrast no significant difference in HDL-C levels were observed in compounds **C101** and **C108** compared to HG-treated rats.

LDL levels:

The elevated plasma LDL levels produced by Triton WR-1339 administration were significantly suppressed (24%, 29%, 28% and 28% $p < 0.001$) in compounds **C110, C103, C104** and **C111** respectively compared to HG. While no significant difference in LDL levels were observed in compounds **C101, C108** and **BF** compared to HG-treated rats.

In summary, a significant decrease in TG and LDL and an increase in HDL plasma levels were observed with compounds **C110, C103, C104** and **C111**. In addition, compounds **C103, C104** and **C111** showed a significant reduction in TC levels too.

Table 9. Effect of removal of benzyl group from indole carboxamides on plasma lipid levels in Triton WR-1339-induced hyperlipidemic rats after 8 hrs.

Lipid profile	TC (mg/dl)	TG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
CG	106.8 ± 1.46	126.7 ± 3.95	48.5 ± 1.12	31.3 ± 1.32
HG	224.6 ± 6.59^{c}	1519.7 ± 20.9^{c}	31.9 ± 1.90^{c}	62.6 ± 1.49^{c}
C103	172 ± 6.29^{c}	441 ± 30.09^{c}	50.4 ± 1.35^{b}	45.1 ± 1.64^{b}
C101	290 ± 29.31	1489 ± 18.8	30.8 ± 2.17	57.3 ± 2.67
C110	240 ± 9.1	506 ± 9.88^{c}	53 ± 0.62^{c}	48.1 ± 1.58^{b}
C104	173 ± 6.0^{c}	477 ± 15.31^{c}	50.7 ± 2.31^{b}	45.6 ± 1.38^{b}
C111	175 ± 4.05^{c}	481 ± 17.06^{c}	51.7 ± 2.11^{b}	45.4 ± 1.45^{b}
C108	262 ± 16.2	1541 ± 11.2	29.7 ± 1.35	60.2 ± 1.55
BF	218.8 ± 5.93	574 ± 17.8^{c}	43.1 ± 1.52^{c}	61.9 ± 1.96

Values are means ± SEM from six rats in each group. CG: normal control group; HG: hyperlipidemic +4% DMSO control group; C103: compound C103+4% DMSO; C101: compound C101+4% DMSO; C110: compound C110+4% DMSO; C104: compound C104+4% DMSO; C111: compound C111+4% DMSO; C108: compound C108+4% DMSO;BF: bezafibrate+4% DMSO; TC: total cholesterol; TG: triglyceride; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol. HG is compared to CG. C103, C101, C110, C104, C111, C108 and BF are compared with HG. ^{b} $p<0.001$, ^{c} $p<0.0001$

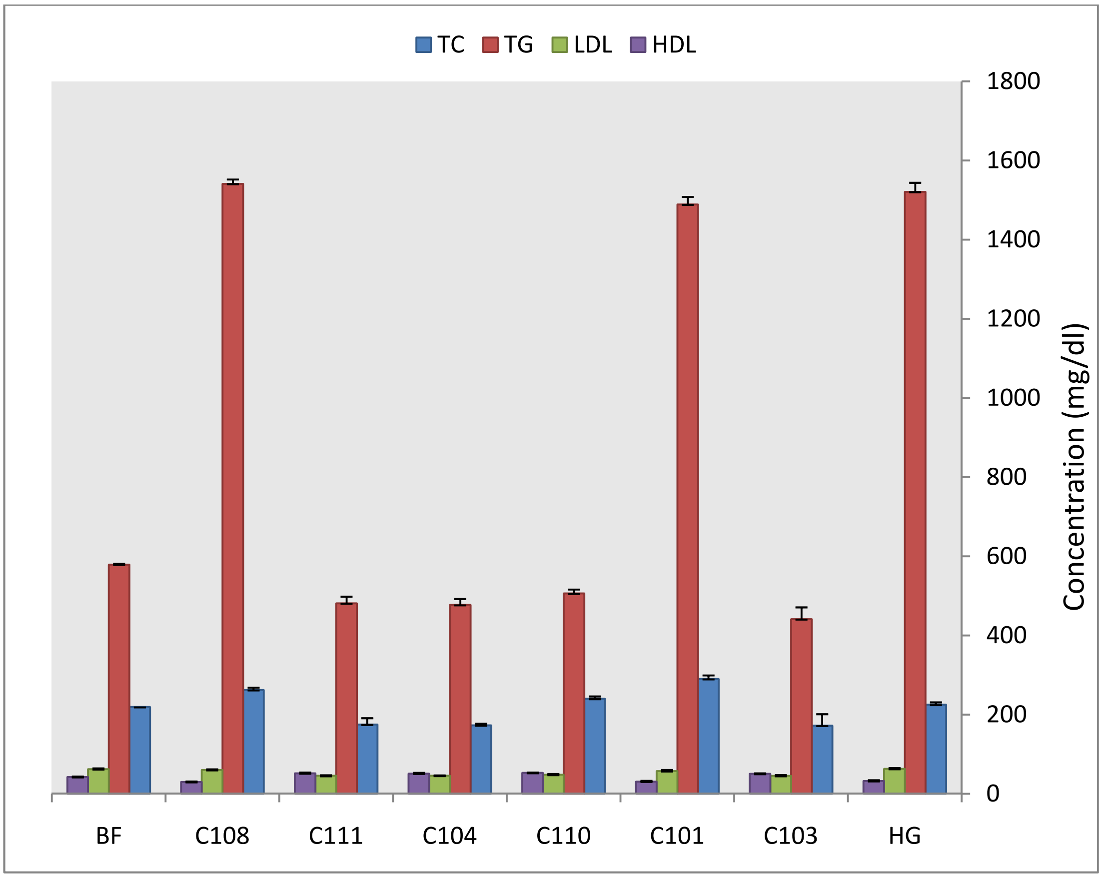


Figure 54. Effect of removal of benzene group from indole carboxamides on lipid profile (mg/dl) after 8 hrs of Triton administration.
Values are means ± SEM from six rats in each group. CG: normal control group; HG: hyperlipidemic +4% DMSO control group; C103: compound C103+4% DMSO; C101: compound C101+4% DMSO; C110: compound C110+4% DMSO; C104: compound C104+4% DMSO; C111: compound C111+4% DMSO; C108: compound C108+4% DMSO;BF: bezafibrate+4% DMSO; TC: total cholesterol; TG: triglyceride; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol. HG is compared to CG. C103, C101, C110, C104, C111, C108 and BF are compared with HG.

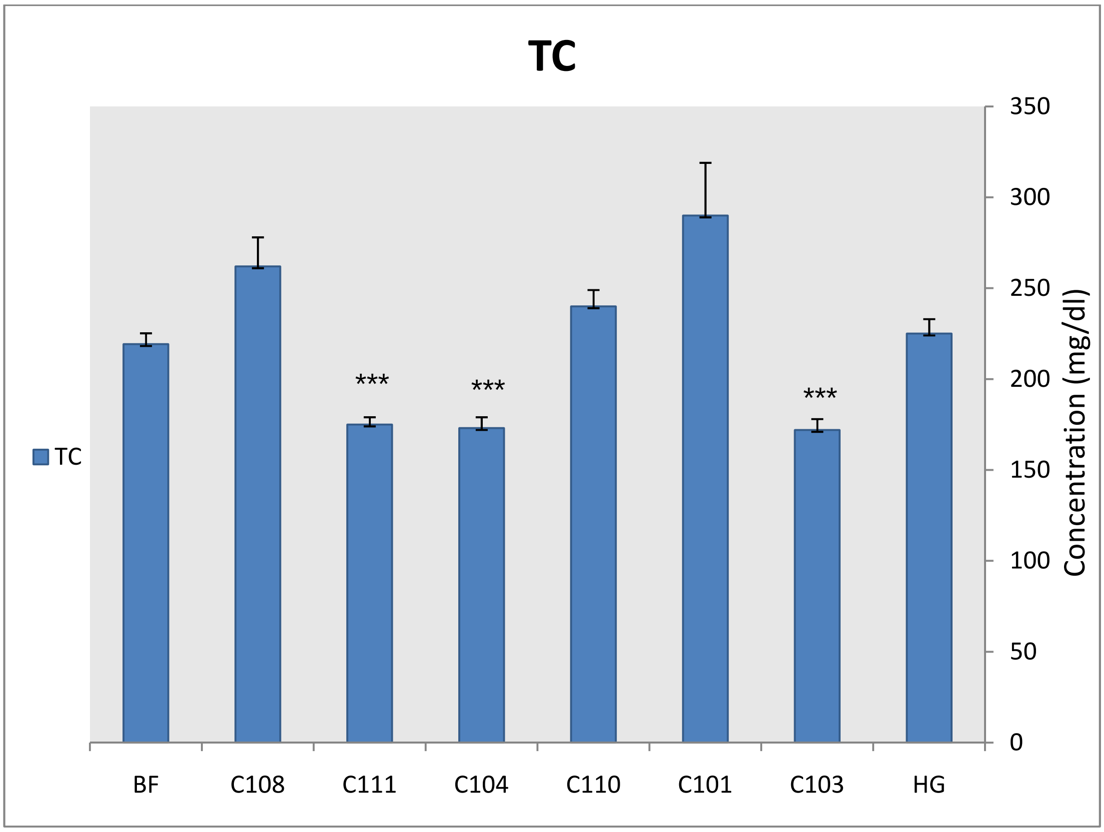


Figure 55. Effect of removal of benzene group from indole carboxamides on total cholestrol (mg/dl) after 8 hrs of Triton administration.

Values are means ± SEM from six rats in each group. CG: normal control group; HG: hyperlipidemic +4% DMSO control group; C103: compound C103+4% DMSO; C101: compound C101+4% DMSO; C110: compound C110+4% DMSO; C104: compound C104+4% DMSO; C111: compound C111+4% DMSO; C108: compound C108+4% DMSO;BF: bezafibrate+4% DMSO; TC: total cholesterol; TG: triglyceride; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol. HG is compared to CG. C103, C101, C110, C104, C111, C108 and BF are compared with HG.

*** $p<0.0001$

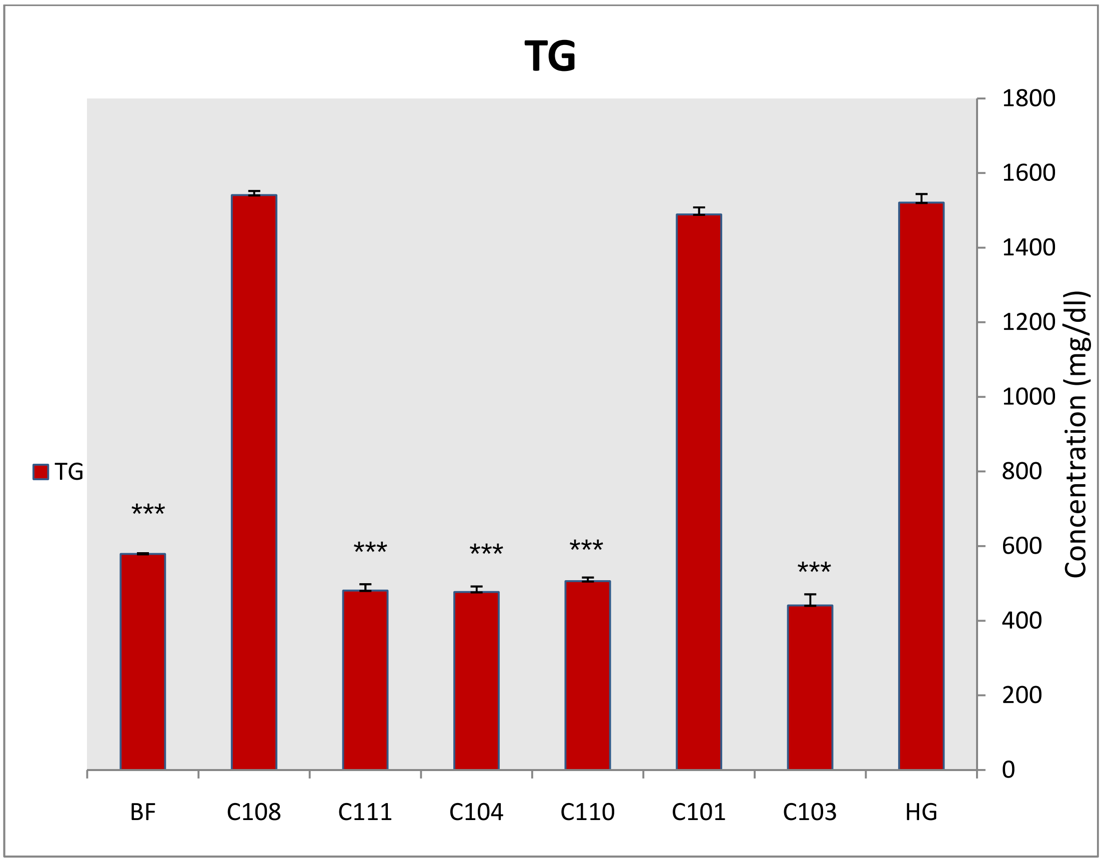


Figure 56. Effect of removal of benzene group from indole carboxamides on triglycerides (mg/dl) after 8 hrs of Triton administration.

Values are means ± SEM from six rats in each group. CG: normal control group; HG: hyperlipidemic +4% DMSO control group; C103: compound C103+4% DMSO; C101: compound C101+4% DMSO; C110: compound C110+4% DMSO; C104: compound C104+4% DMSO; C111: compound C111+4% DMSO; C108: compound C108+4% DMSO;BF: bezafibrate+4% DMSO; TC: total cholesterol; TG: triglyceride; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol. HG is compared to CG. C103, C101, C110, C104, C111, C108 and BF are compared with HG.

***$p<0.0001$

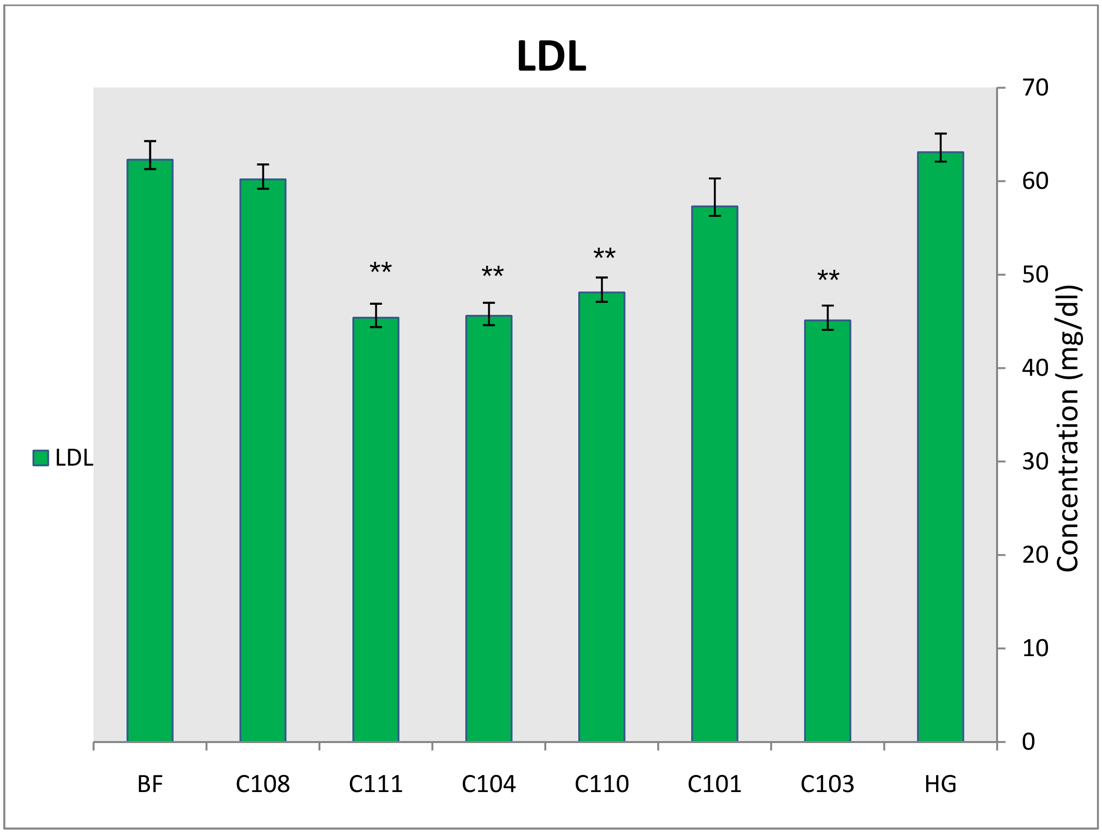


Figure 57. Effect of removal of benzene group from indole carboxamides on low-density lipoproteins (mg/dl) after 8 hrs of Triton administration.
Values are means ± SEM from six rats in each group. CG: normal control group; HG: hyperlipidemic +4% DMSO control group; C103: compound C103+4% DMSO; C101: compound C101+4% DMSO; C110: compound C110+4% DMSO; C104: compound C104+4% DMSO; C111: compound C111+4% DMSO; C108: compound C108+4% DMSO;BF: bezafibrate+4% DMSO; TC: total cholesterol; TG: triglyceride; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol. HG is compared to CG. C103, C101, C110, C104, C111, C108 and BF are compared with HG.

** $p<0.001$

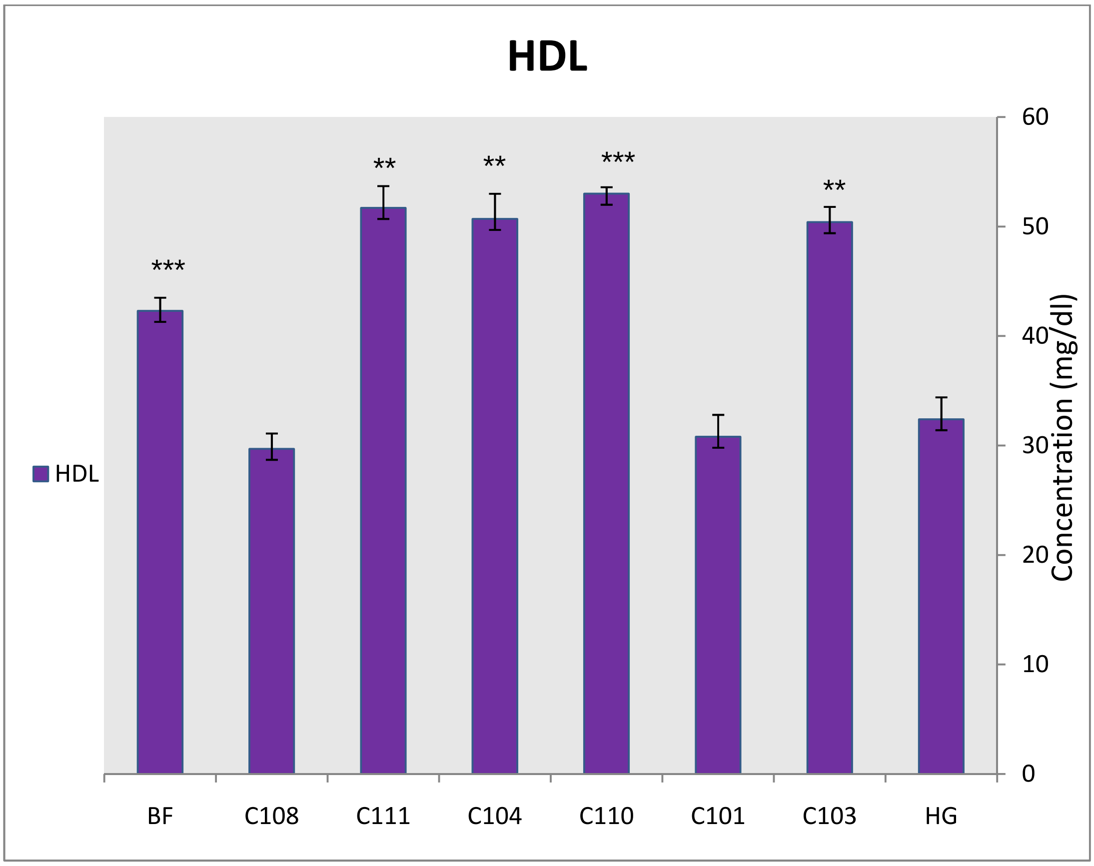


Figure 58. Effect of removal of benzene group from indole carboxamides on high-density lipoproteins (mg/dl) after 8 hrs of Triton administration.

Values are means ± SEM from six rats in each group. CG: normal control group; HG: hyperlipidemic +4% DMSO control group; C103: compound C103+4% DMSO; C101: compound C101+4% DMSO; C110: compound C110+4% DMSO; C104: compound C104+4% DMSO; C111: compound C111+4% DMSO; C108: compound C108+4% DMSO;BF: bezafibrate+4% DMSO; TC: total cholesterol; TG: triglyceride; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol. HG is compared to CG. C103, C101, C110, C104, C111, C108 and BF are compared with HG.

** $p<0.001$, *** $p<0.0001$

8.3. Biological Discussion:

The results of this study demonstrated the potential hypolipidemic effect of 1*H*-indole-2-carboxamide derivatives (Table **2, 3, 4, 5, 6, 7, 8** and **9**) in Triton WR-1339 induced hyperlipidemic rats.

These observations could be explained by understanding the mechanism through which Triton WR-1339 induces hyperlipidemia in rats. It has been reported that a single parenteral administration of Triton WR-1339 to adult rats produces hyperlipidemia in which cholesterol, triglycerides and phospholipids increase to a maximum in about 12 hrs and decrease thereafter (Bittner *et al.*, 2009; Gaziano *et al.*, 1997). Triton WR-1339 acts as surfactant, suppresses the action of lipase and blocks the uptake of lipoproteins from the circulation by extrahepatic tissues resulting an increase in the levels of circulating lipid (Sashidhara, 2008). The large increase in plasma cholesterol and triglycerides due to Triton Wr-1339 injection results mostly from an increase of VLDL secretion by the liver accompanied by a strong reduction of VLDL and LDL catabolism (Gammill, 2003).

In our hands, this model gave similar plasma lipid profile changes (TG), after Triton WR-1339 injection in rats (Figure 59). This result demonstrates the feasibility of using Triton induced hyperlipidemic rats as an experimental model to investigate the hypolipidemic effect of our investigated compounds.

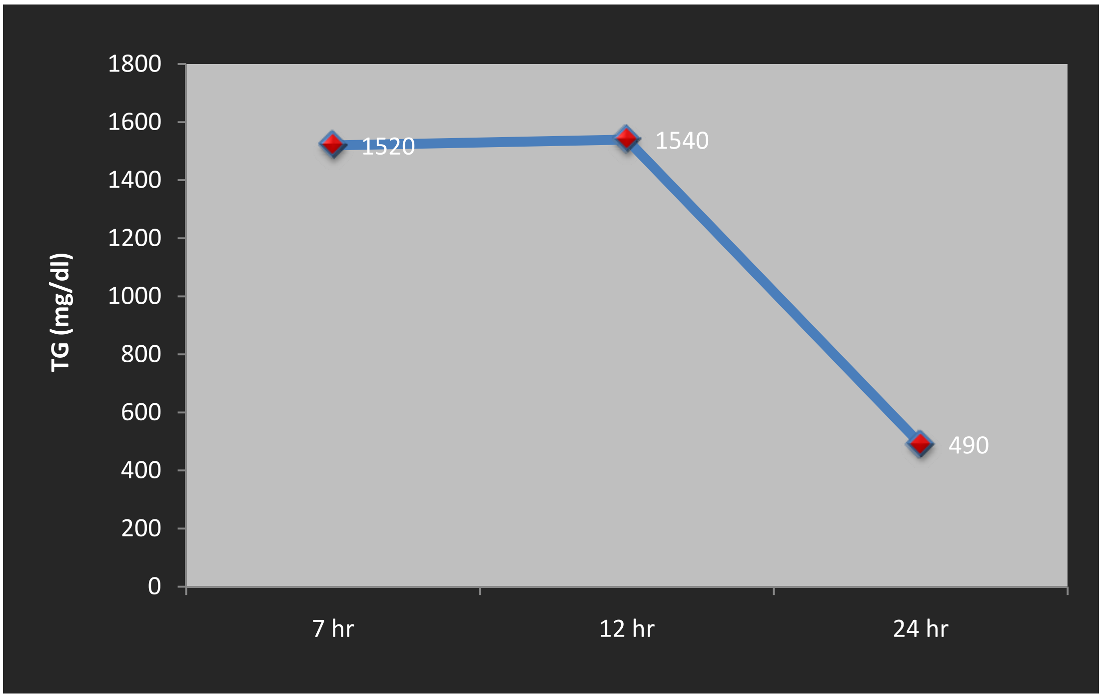


Figure 59. The effect of Triton WR-1339 administration to adult rats, on triglycerides levels over 24 hours.

The reduction of total cholesterol by compounds **6, 18, 24, 26, 36, 88, 90, 103, 104** and **109**, was associated with a decrease of its LDL fraction, which is the target of several hypolipidemic drugs. This result suggests that cholesterol-lowering activity of these compounds can be result from the rapid catabolism of LDL cholesterol through its hepatic receptors for final elimination in the form of bile acids (Venkateswarulu *et al*., 2010).

Clearly, our results showed that compounds **6, 10, 18, 22, 24, 26, 36, 38, 88, 90, 93, 110, 103, 104** and **111**, at 57 µM, were able to significantly decrease serum triglycerides levels. Thus, since the proportion of triglyceride in VLDL is many times higher than cholesterol, it is not surprising that the hypolipidemic activity of these compounds was significantly higher for triglycerides than for cholesterol. This result suggests that our compounds are able to restore, at least partially, catabolism of apo-B

lipoproteins as hypothesized by many studies with other lipid-lowering agents (Gotto and DPhil, 2002).

In addition, compounds **6, 10, 18, 22, 24, 26, 36, 38, 88, 90, 93, 110, 103, 104** and **111,** increased HDL level, which is known for its preventive role against atherogenesis. HDL plays an important role in facilitating the mobilization of triglycerides and cholesterol from plasma to liver where it undergoes catabolism and then eliminated in the form of bile acids (Beck *et al*., 2004).

The underlying mechanism of this activity is hypothesized by many works with other drugs, that the catabolic restoration of catabolic metabolism of TG's could be due to an increased stimulation of LPL (Alper *et al*., 2009; Venkateswarulu *et al*., 2010). The large decrease in plasma HDL-C levels due to Triton WR-1339 injection results mostly from a progressive displacement of the apo A-1 protein from the HDL surface, without loss of lipid (Beyer *et al*., 2008).

PPARα is a nuclear receptor that plays an important role in lipid homeostasis. Besides its major role in regulating Apo CIII, apo AV and fatty acid oxidation, it has been well recognized that PPARα agonists regulate key molecular components involved in HDL cholesterol metabolism (Mori *et al*., 2007). Specifically, PPARα agonists upregulate human ApoAI. Apo AI is the major structural component of HDL particles and the amount of Apo AI is closely associated with HDL cholesterol levels (Beyer *et al*., 2008).

Furthermore, a more recent study revealed that fibrates are not selective agonists of PPARα and their effects on other isoforms of PPARs are rather complex. Therefore our target compounds has more propability to act as selective PPARα agonist (Koyama *et*

al., 2005). These data are supported by their effect on HDL compared to BF, this suggestion needs further investigation.

Mean while the large increase in plasma TG levels due to Triton administration results mostly from an increase of VLDL secretion by the liver accompanied by strong reduction of VLDL and LDL catabolism.

Promisingly, the reduction of lipid profile by compounds **6, 10, 18, 22, 24, 26, 36, 38, 88, 90, 93, 110, 103, 104** and **111**, at 57 µM after 8h of Triton injection is more significant than the reduction induced by bezafibrate at (276 µM, 100 mg/kg body weight), which in this study has been used as standard reference hypolipidemic drug. Furthermore, total cholesterol levels were not significantly changed which agrees with the mechanism of action of fibrates in that their total cholesterol-lowering activity is not strongly marked, but the triglycerides decreasing effect of them is very impressive especially by stimulation of the gene expression of lipoprotein lipase (Alper *et al*., 2009).

8.3.1 Preliminary SAR:

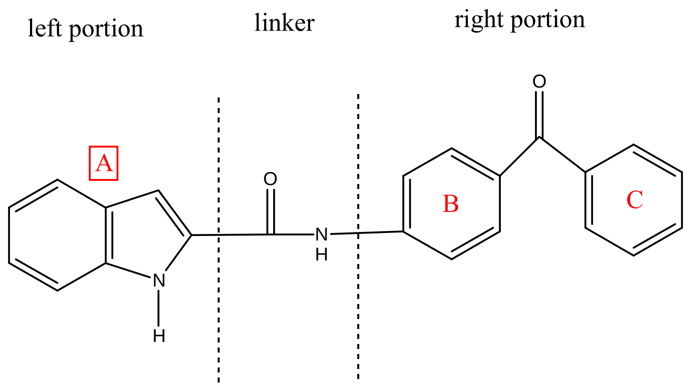


Figure 60: Compound **6** an initial hit and SAR strategy

Herein, we describe the preliminary structure–activity relationship (SAR) studies on this novel synthetic agents, as a potential LPL agonists. Strategically, compound **6** was identified as an initial hit that showed an excellent antihyperlipidemic activity *in-vivo* in compare to a reference drug. Compound **6** was divided into three portions: an indole moiety (left portion), carboxamide (linker), and benzophenone (right portion), and systematic SAR studies were conducted in each portion (Figure 60).

Therefore, in terms of SAR, the minimum structural requirement for binding of the derivatives to the receptor includes:

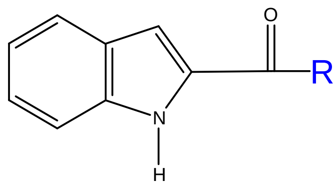
1. A heteroaromatic ring (ring A), which is believed to participate in H-bonding (donor or acceptor) with the amino acid residue of the receptor, which is represented by indol, pyrrole and furan ring.

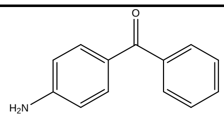
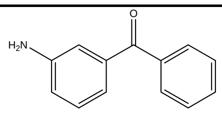
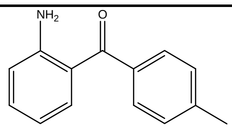
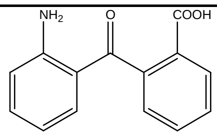
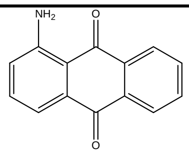
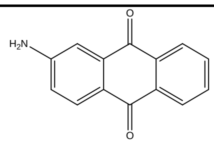
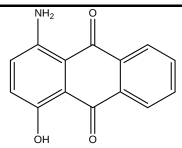
2. A carboxamide linker, which is believed to participate in H-bonding (donor or acceptor) with the amino acid residue of the receptor.

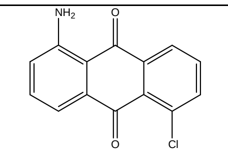
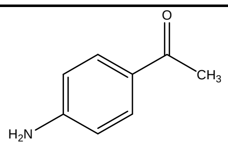
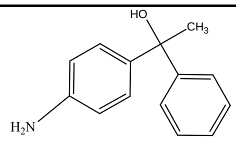
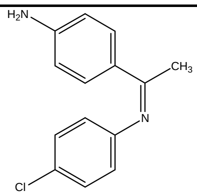
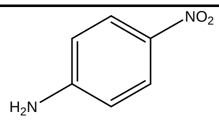
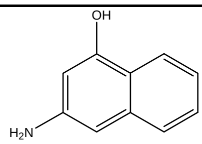
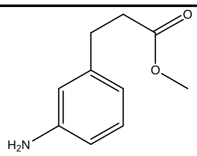
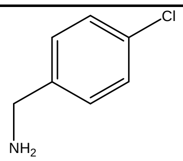
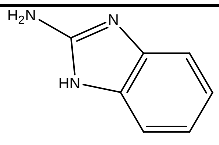
3. Lipophilic rings (ring B & C), which is believed to participate in π– π stacking with aromatic amino acids residue of the receptor, and add in the lipophilicity of the compound.

4. An extended form of the target structures, is critical to provide an active hit.

Table 10: SAR of the right portion with different substitution.



Compoud	R	TC[a](mg/dl)	TG[a](mg/d)	HDL[a](mg/d)	LDL[a](mg/d)
6		**178**	**435**	**52.1**	**46.4**
10		**234**	**440**	**51.3**	**46.7**
14		**244**	**1522.3**	**28.5**	**66.3**
16		**227.7**	**1523.3**	**29.8**	**59.2**
18		**179**	**529**	**52.6**	**48.1**
20		**272**	**1523**	**28.3**	**65.4**
22		**228**	**456**	**52.4**	**45.3**

24		**180**	**452**	**51.8**	**44.9**
28		**263**	**1534**	**27.4**	**67.3**
36		**196**	**1172**	**59.7**	**66.2**
38		**185**	**672**	**56.5**	**53.4**
51		**272**	**1526**	**26.2**	**65.8**
57		**255**	**1529**	**28.7**	**66.8**
63		**258**	**1530**	**28.3**	**64.3**
67		**260**	**1539**	**28.2**	**67.2**
83		**278**	**1521**	**31.5**	**62.5**

[a]Values are means ± SEM from six rats in each group

Initial SAR studies indicated that the presence of two phenyl group (B & C) is essential for activity (compare **6** and **18** with **28**, Table 10). Ring (B & C) did not only provide π-π stacking, but also lipophilic effect that was essential for active hit. Carbonyl group between (B & C) seems to be favorable but not essential. An isoster replacement to carbonyl maintained the activity (compound **36** and **38**), but to a lower extent. On the other hand lipophilicity is critical because of hydration problem of the receptor, since a hydrophilic moiety would affect the activity (compare **6** and **83**). This theory was supported by previous work performed by our research team (Abu Sheikha *et al*., 2011) in which, they discovered that benzothiophene; a lipophilic group; linked to another lipophilic group such as benzophenone or anthraquinone was inactive. However, when benzothiophene linked with pyridine or benzimidazole, which gave the hydrophilic part of the molecule, produced an active hit, this phenomena was termed reverse amide hypothesis. These results implicated that for a hit to be active, a lipophilic and a hydrophilic part is required in the same molecule, without regard to its position whether on the left portion or on the right portion of the hit. Substitution on the active compounds (right portion) with electron donating or withdrawing group is tolerable (compare **22** and **24**). An important point in these targets, is the shape of the targets or its spatial configuration, an extended form is active, while a V-shaped compound is inactive. The attachment point of the amide linker seems to play a critical role. Switching the amide linker for example, from the 4-or 3-position in compound **6** to the 2-positions, was detrimental to the activity (compare **6** , **10** with **14**).

It is worth to implicate that the finding of this research indicates that lipid-lowering active compounds for example **(6** and **10)** require an extended form, where as non-active compound (**14**), requires non-extended form. These suggestions were supported

by Energy-Minimized Structures for compounds **6** and **14** as showed in (Figure 60). Such orientation prevents attacking of the compound to the receptor making it inactive.

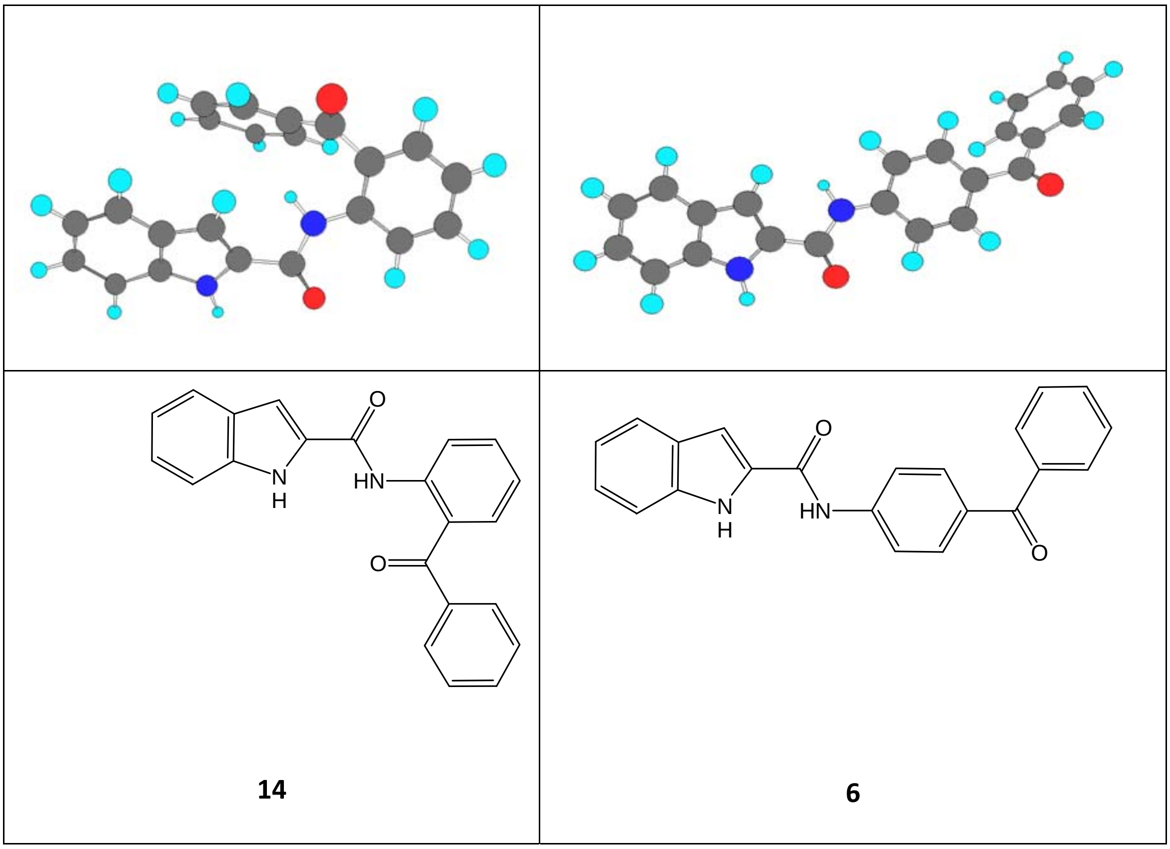


Figure 61. 3-D Energy Minimized Structures of benzophenone derivatives (compounds **6** and **14).**

Figure 61 shows that **C6** exhibited an extended structure. Apart from the last benzophenone phenyl group, the structure also exhibited a linear form. On the other hand, **C14** exhibited a non-extended non-linear, V-shaped structure. This 3D-orientation makes the NH of the amide and the carbonyl group of the benzophenone in **C16** in close proximity, possibly permitting intra-molecular hydrogen bonding.

The same with anthraquinone derivatives, (Figure 61 and 62) show the Energy-Minimized Structures for compounds **18** and **20.** The same conclusion regarding their

spatial configuration was attained for the active and non-active compounds. Compound **18** the active one exhibited and extended orientation, while the inactive **C20** exhibited a non-extended orientation.

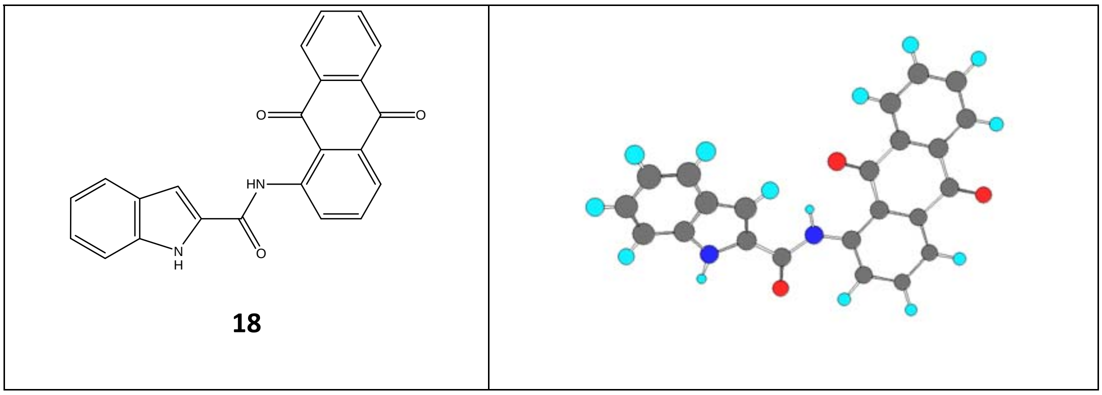


Figure 62. 3-D Energy Minimized Structures (ChemDraw Ultra 8.0) of anthraquinone derivatives **C18**.

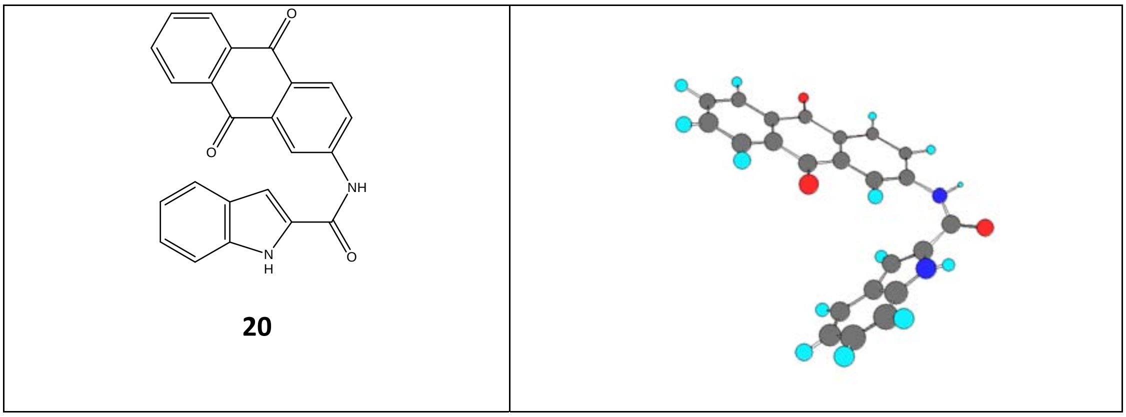
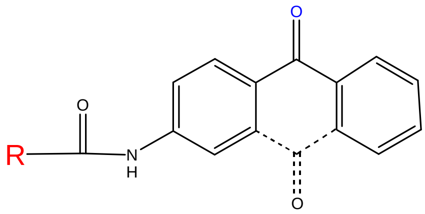
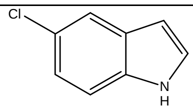
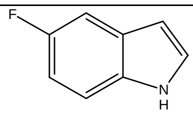
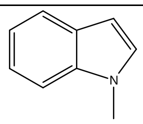
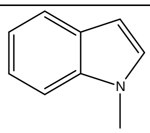
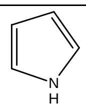
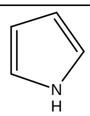
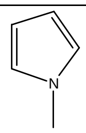
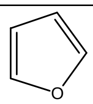
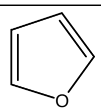


Figure 63. 3-D Energy Minimized Structures (ChemDraw Ultra 8.0) of anthraquinone derivative **C20**.

Table 11: SAR of the left portion of the active hits with different substitution.



Compound	**R**	**TC[a] (mg/dl)**	**TG[a](mg/dl)**	**HDL[a](mg/d)**	**LDL[a] (mg/dl)**
88		176	442	52.2	45
93		187	433.8	53.2	44
96		246	1533	28.8	66.5
97		278	1585	29.1	64.3
103		172	441	50.4	45.1
104		173	477	50.7	45.6
108		262	1541	29.7	60.2
110		240	506	53	48.1
111		175	481	51.7	45.4

[a]Values are means ± SEM from six rats in each group

As indicated in (Table 11), removal of the fused benzene ring, has no effect on the activity, indicating that its presence is not essential for activity (compare **103** in table 2 with **6** in table 1). Substituent on the left portion indole of the targets, have no effect on the binding of the targets to the receptor and subsequently on their activity as dyslipidemic agents (compare **88** and **93** in table 2 with **6** in table 1). No activity was seen with the N-methylation of the heterocyclic group (compare **103** with **108** and **97** in table 2 with **18** in table 1) implying the requirement of H-bonding at this site for binding.

This was supported with compounds (**110** and **111**). In which the N was replaced with O, indicated that either H-bond donor or acceptor is needed for binding with the receptor. This final conclusion is supported by previous data regarding isosteric replacement of indole. Isosteres 4-benzofuran 2-carboxamide (**A**) was reported to be active, where as benzothiophene 2-carboxamide (**B**) was inactive (Figure 63) (Shattat *et al*., 2010; Abu Sheikha *et al*., 2011).

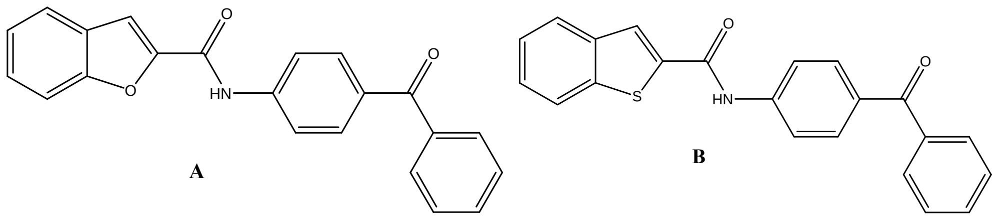


Figure 63. The chemical structure for 4-benzofuran 2-carboxamide (**A**) and benzothiophene 2-carboxamide (**B**)

9. Experimental model of *in-vitro* antioxidants:

Antioxidant activity was determined based on the ability of the antioxidants to act as radical scavengers towards the stable free radical, that is based on the reduction of 1,1-diphenyl-2-picrylhydrazyl (DPPH) (Figure 64). Due to the presence of an odd electron it gives a strong absorption maximum at 517 nm. As this electron becomes paired off in the presence of a hydrogen donor, i.e. a free radical scavenging antioxidant, the absorption strength is decreased, and the resulting decolorization is stoichiometric with respect to the number of electrons captured (Jothy *et al*., 2011; Al-Laith, 2010).

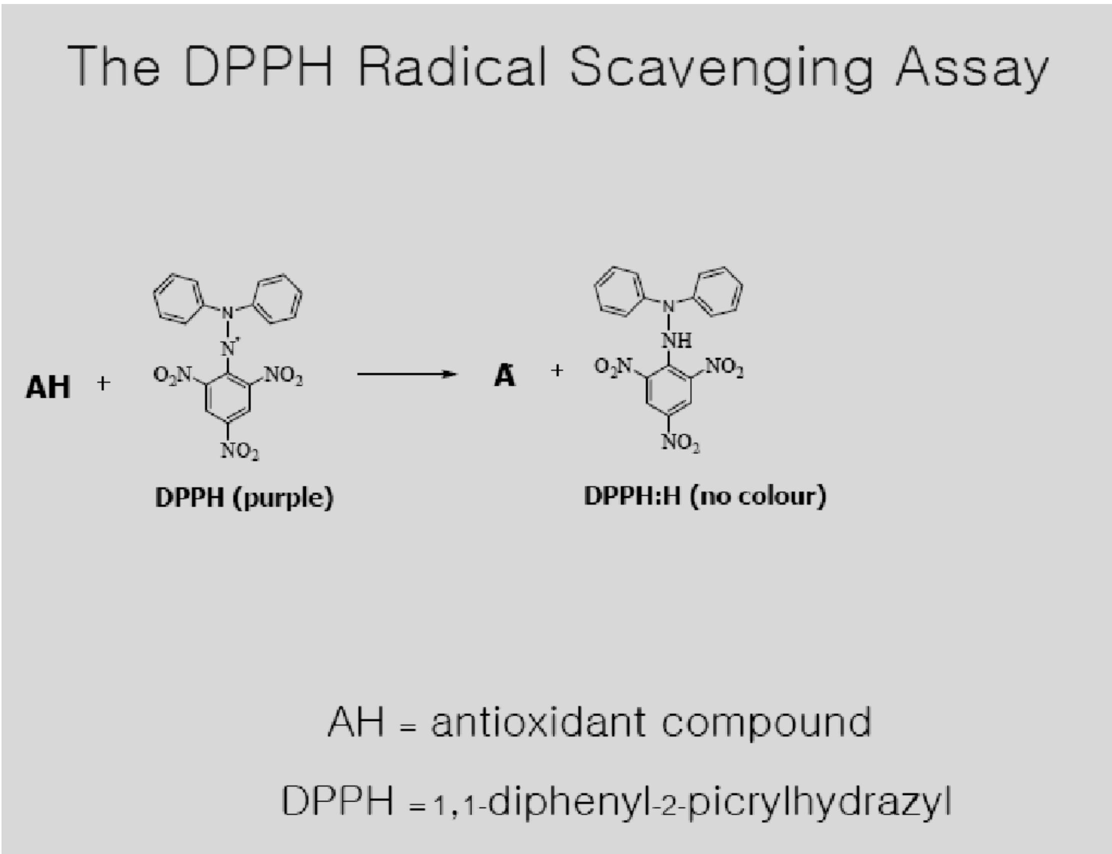


Figure 64. The DPPH radical scavenging assay.

9.1. Materials and methods:

DPPH was purchased from Sigma–Aldrich (USA). Stock solutions of DPPH were prepared in methanol and DMSO. The solvents and other chemicals were of analytical grade. The reaction tubes, in triplicate, were wrapped in aluminum foil and kept at 30 °C for 30 min in dark. All measurements were carried out under dim light. Spectrophotometric measurements were obtained at 517 nm using Spectronic Genesys 5 spectrophotometer. Data were expressed as mean ± standard deviation (x ± SD).

9.2. Experimental protocol:

The DPPH assay was used to measure the ability of the compounds to scavenge the stable free radical of DPPH spectrophotometrically. DPPH (0.002%) solution in methanol was prepared. Then 1 ml of this solution was mixed with 1 ml of sample solution (20 µg/ml in methanol and DMSO, 9:1). The blank was used as 1 ml of methanol and DMSO (9:1) with 1 ml of DPPH solution (0.002%). After 30 minutes of incubation in dark at room temperature, the absorbance was observed against a blank at 517 nm. Ascorbic acid (V.C) was used as positive control, with calculated IC_{50} = 0.5 µg/ml. The assay was carried out in triplicate and the percentage of inhibition was calculated using the following formula (Jothy *et al*., 2011):

% Inhibition = [(AB – AA) / AB] x 100

Where, AB = absorption of blank and AA= absorption of test compounds

9.3. Results and discussion:

DPPH is a stable free radical in a methanolic solution. In its oxidized form, the DPPH radical has an absorbance maximum centered at about 520 nm. The DPPH method is described as a simple, rapid and convenient method independent of sample polarity for screening of many samples for radical scavenging activity. These advantages make the DPPH method interesting, for screening test of synthetic targets to scavenge radicals and to find out promising candidates as antioxidants (Sharma and Bhat, 2009; Marxen *et al*., 2007).

Primary antioxidants (AH), when present in trace amounts, act as chain-breaking antioxidants by either delaying or inhibiting the initiation step, by reacting with peroxyl or alkoxyl radicals, as shown below:

$R^{\cdot} + AH \rightarrow RH + A^{\cdot}$

$ROO^{\cdot} + AH \rightarrow ROOH + A^{\cdot}$

$RO^{\cdot} + AH \rightarrow ROH + A^{\cdot}$

The antioxidant free radical may further interfere with the chain-propagation reactions by forming peroxy antioxidant compounds:

$A^{\cdot} + ROO^{\cdot} \rightarrow ROOA$

$A^{\cdot} + LO^{\cdot} \rightarrow LOA$

This free radical scavenging capacity of the target compounds, is therefore attributed to their hydrogen-donating capacity due to the presence of hydroxyl groups in their structure (Atmani *et al*., 2009; Wanasundara and Shahidi, 2005).

In the present work, a series of selected new synthesized heterocyclic carboxamide derivatives were screened for their antioxidant activity by DPPH radical scavenging cation decolorizaton assays. Their structure- activity studies are summarized in (Table 12) and (Figures 65, 66, 67, 68 and 69). In the present investigation, the compounds **22,**

57, 30, 55 and **59** showed excellent percent inhibition of DPPH activity (82%, 69.3%, 62.5%, 54.7% and 34.3% respectively) and were the most effective DPPH radical scavengers.

Table 12: Antioxidant activity of synthesized heterocyclic carboxamide derivatives (DPPH assay).

compounds	DPPH % Inhibition of 20 μg/ml of the compound *
C59	(34.32 ± 0.33)
C41	(13.76 ± 0.21)
C57	(69.34 ± 1.41)
C28	(11.11 ± 0.10)
C12	(10.41 ± 0.44)
C47	(16.88 ± 1.53)
C83	(26.44 ± 1.00)
C39	(15.89 ± 1.78)
C30	(62.45 ± 1.71)
C45	(10.55 ± 0.44)
C63	(15.33 ± 0.20)
C110	(2.39 ± 0.37)
C67	(11.39 ± 0.10)
C22	(82 ± 2.50)
C10	(23.49 ± 0.49)
C98	(26.02 ± 0.69)
C32	(12.38 ± 0.28)
C55	(54.7± 1.42)
C81	(10.75 ± 0.20)

*Values are of the mean ± SD

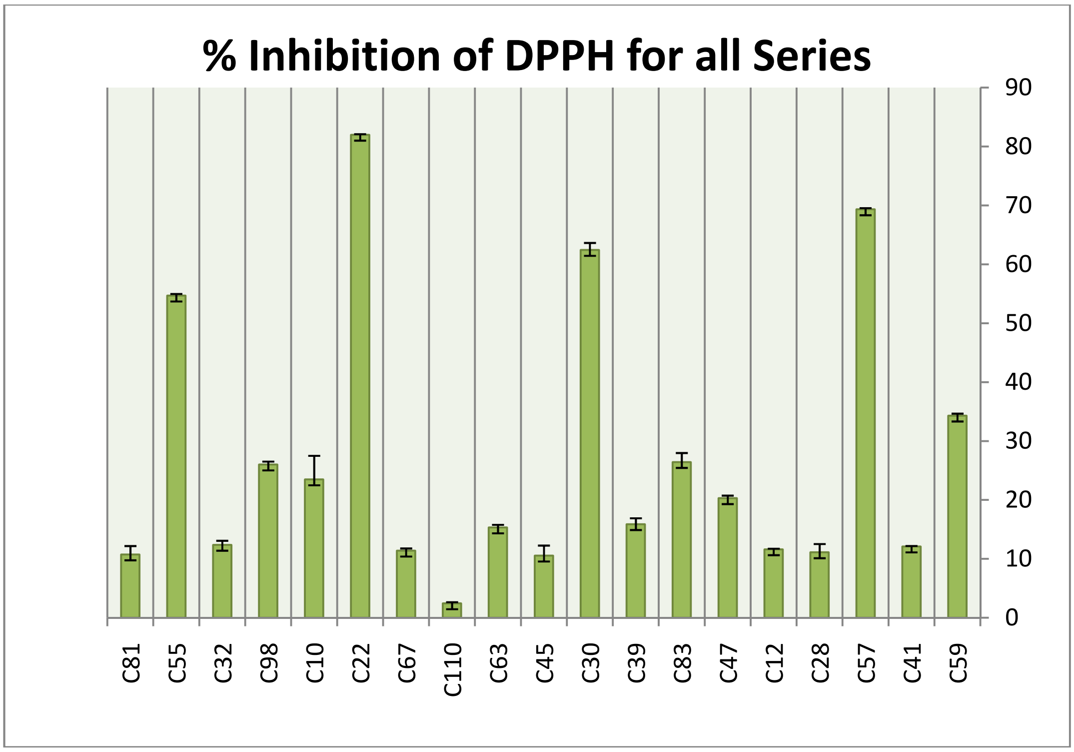


Figure 65: Antioxidant activity of synthesized heterocyclic carboxamide derivatives (DPPH assay).

The synthesized compounds of series D have been evaluated for their antioxidant activity by DPPH assay (Figure 66 and 67). The synthesized compound **57** had shown significant antioxidant activity, which is attributed to the presence of heterosystem (fused benzene with OH group). The formed antioxidant radical is stabilized by delocalization of the unpaired electron around the phenol ring to form a stable resonance hybrid (Wanasundara and Shahidi, 2005). Compound **55** also had a phenol group, hydroxyl groups may exert a great influence on those mechanisms underlying antioxidant activity, due to their ability to neutralize hydroxyl and related free radicals (Ochani and Mello, 2009). In fact, the scavenger capacity of **C57** and **C55** is attributed, in a large extent, to the high reactivity of their hydroxyl groups towards free radicals. Along with, it was observed that the presence of substituents such as COOH, OCH3, Cl, and F also play an important role in deciding antioxidant activity (Wanasundara and Shahidi, 2005). Phenolic acids as in compound **59** serve as free

radical acceptors and chain breakers (Wanasundara and Shahidi, 2005). It was observed from this series that the presence of di-chloro group in the benzene ring reduced the antioxidant activity significantly, may be because of its steric effect.

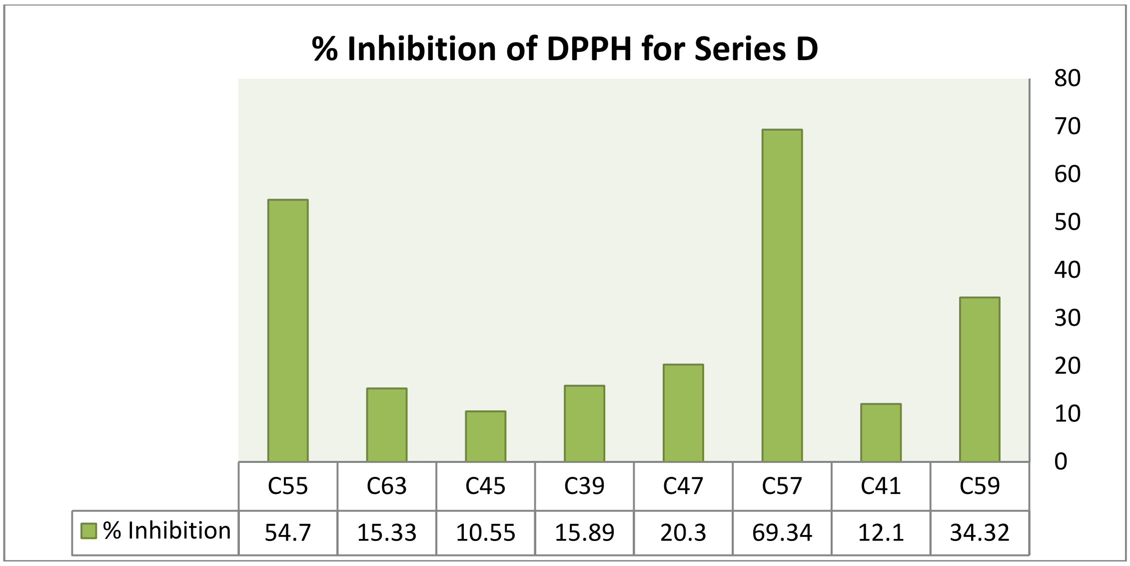


Figure 66: The antioxidant activity of Series D (DPPH assay).

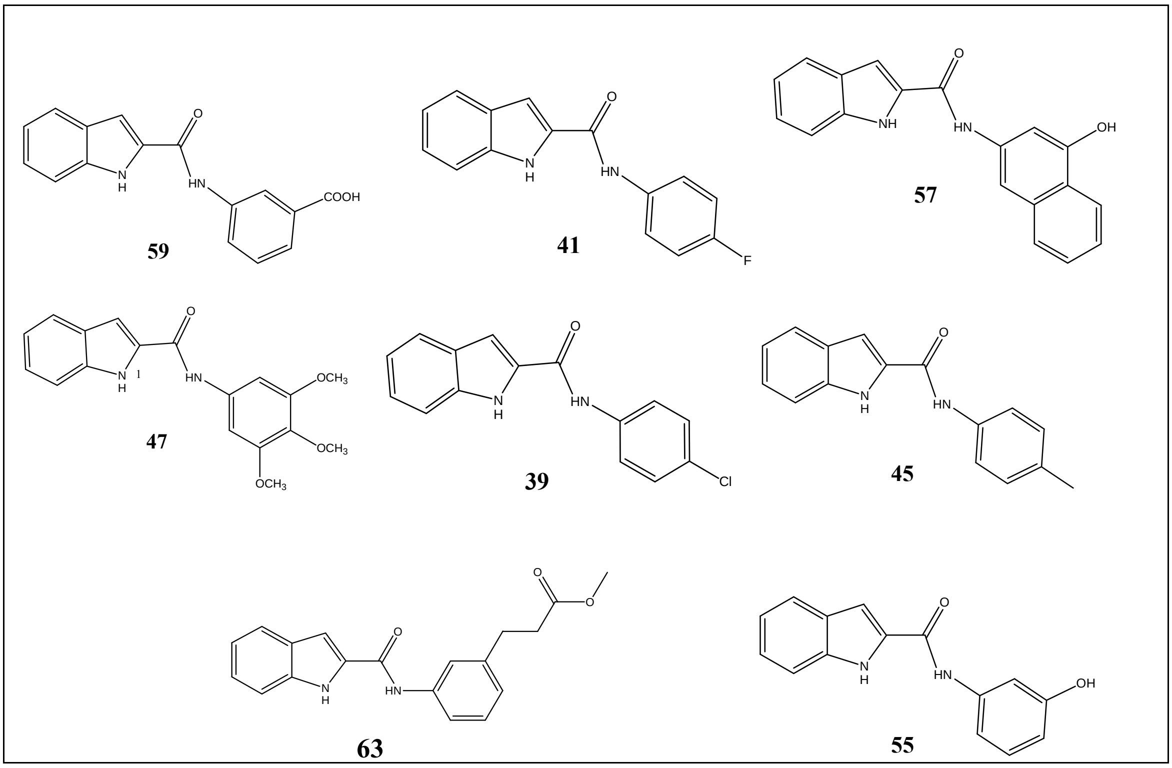


Figure 67: The Chemical structures of compounds of Series D.

Clearly shown in (Figure 68 and 69), replacing Br in compound **65** to Cl in compound **67** enhanced the antioxidant activity dramatically, which indicates the importance of Cl substituent for activity, the mechanism behind this effect in not well known. It is obvious from compound **81** and **83**, that heterocyclic fused ring, provides an electron conjugation and dislocation, thus permitting stability of the radical activity, especially seen in benzimidazole substituent in compound **83** (Wanasundara and Shahidi, 2005; Ochani and Mello, 2009).

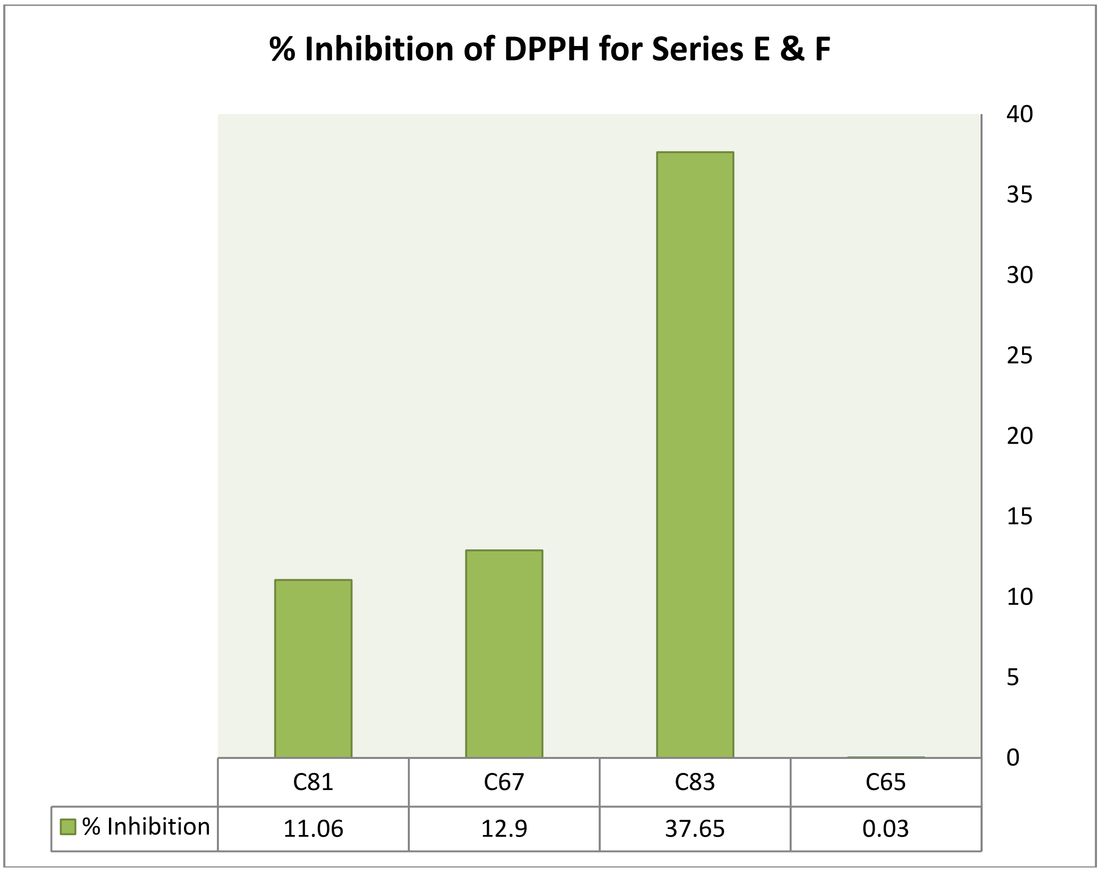


Figure 68: The antioxidant activity of Series E and F (DPPH assay).

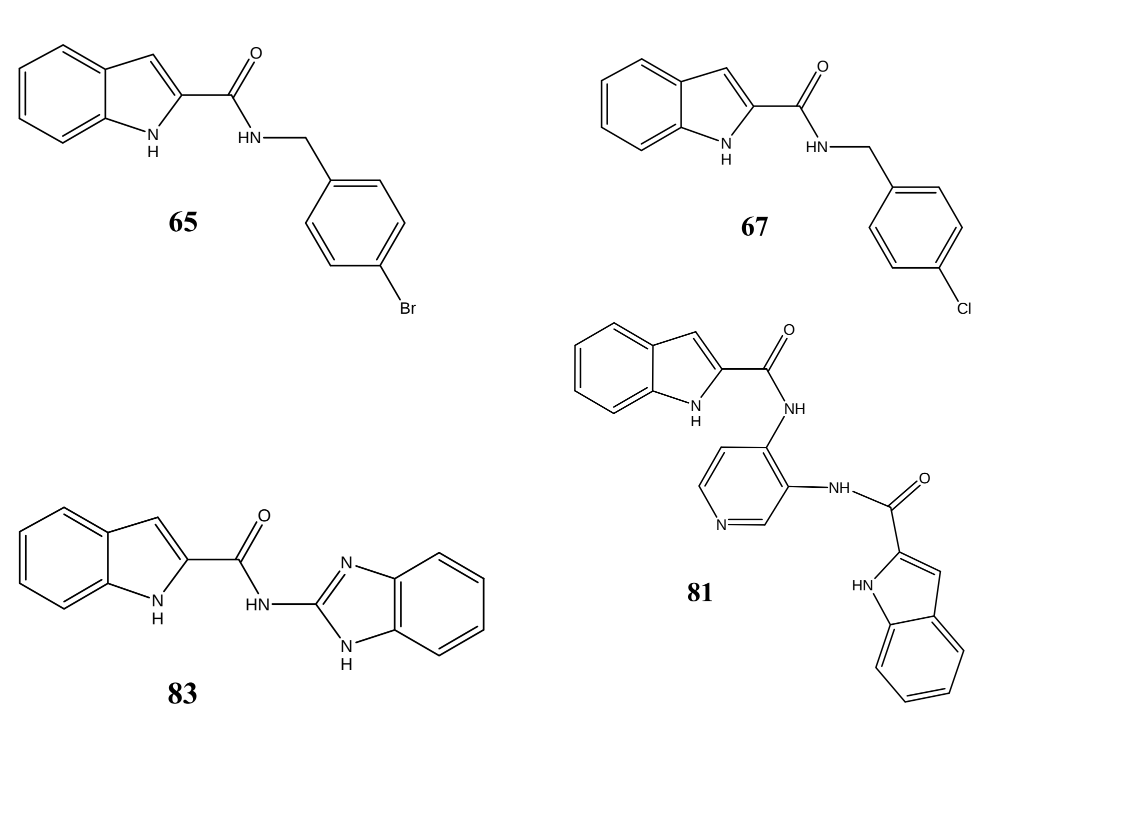


Figure 69. The Chemical structures of compounds of Series E and F.

According to the results in Figure 70 and 71 from series C, **C30** had the strongest antioxidant capability in compare to **C28** and **C32**, which implicated the importance of the position of the amine linked to the acyl group; the planarity of the compound (Atmani *et al*., 2009). DPPH is a comparatively stable free radical at room temperature, it is not water soluble and the reaction mechanism between the antioxidant and DPPH radical depends on the structural conformation of the antioxidant (Wanasundara and Shahidi, 2005). It was found that the meta position of the aminoacetophenone dramatically increased the antioxidant activity.

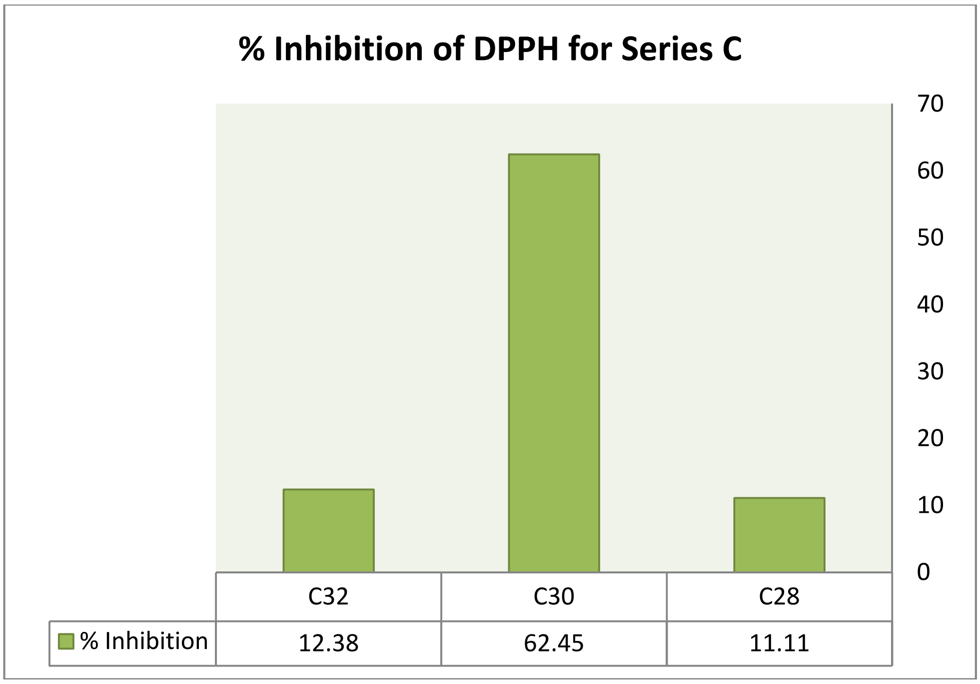


Figure 70: The antioxidant activity of Series C (DPPH assay).

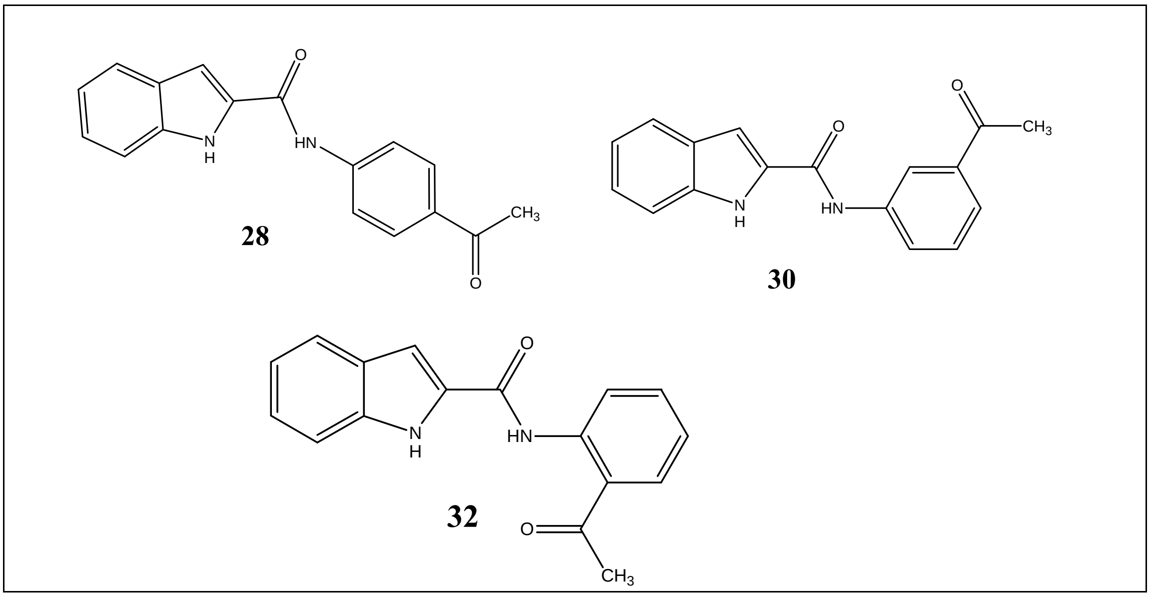


Figure 71: The Chemical structures of compounds of Series C.

Again the planarity of the compounds (the meta position of the aminobenzophenone) linked to the acyl group, is critical for the antioxidant activity (see **C10** and **C98** in comparison to **C12**) (Atmani *et al*., 2009). Another important point deduced from the results in (Figure 72 and 73), that indole ring is essential for antioxidant activities (compare **C110** and **C10**), since it donates a H atom, can act as free radical acceptors/scavengers and delay or inhibit the initiation step or interrupt the propagation step of autoxidation (Lakshmi *et al*., 2010). Although, it is seen that N-methylation of indole ring is tolerable for its antioxidant activity, but to lower extent.

Therefore, the indole nitrogen is the active redox center of indoles. The indole nucleus is already reported as the reactive center towards radical species, and this reactivity has been attributed to its high resonance stability and very low energy barrier towards radical reactions (Estevão *et al*., 2010).

Compound **22**, showed a significant increase in the radical scavenging activity. In addition, this compound showed an excellent dyslipidemic activity too, which could be a promising candidate for a novel compound with dual activity (antihyperlipidemic and antioxidant activity).

Other compounds such as compounds **102, 24, 20, 104, 111, 103, 104, 105, 97, 77, 26, 18, 14, 6, 8, 16, 43, 73, 71, 75** and **61** have been tested using DPPH assay method. Compounds **14, 6, 8, 43, 73, 71, 75** and **61** showed no significant difference in their antioxidant activity compared to blank, this could be explained by the effect of substitutions and their position in the indole nucleous created a minor distortion in the structure, suppressing structural planarity and electron conjugation and thereby compromising scavenger capacity (Atmani *et al*., 2009).

However, compounds **102, 24, 20, 104, 111, 103, 104, 105, 97, 77, 26, 18** and **16** their absorbance were not attainable, because of solubility problems, therefore we excluded them from the results. Therefore, a modified procedure need to be conducted in order to measure their activity, to make better conclusion on the SAR of these novel synthesized compounds in this research as antioxidants.

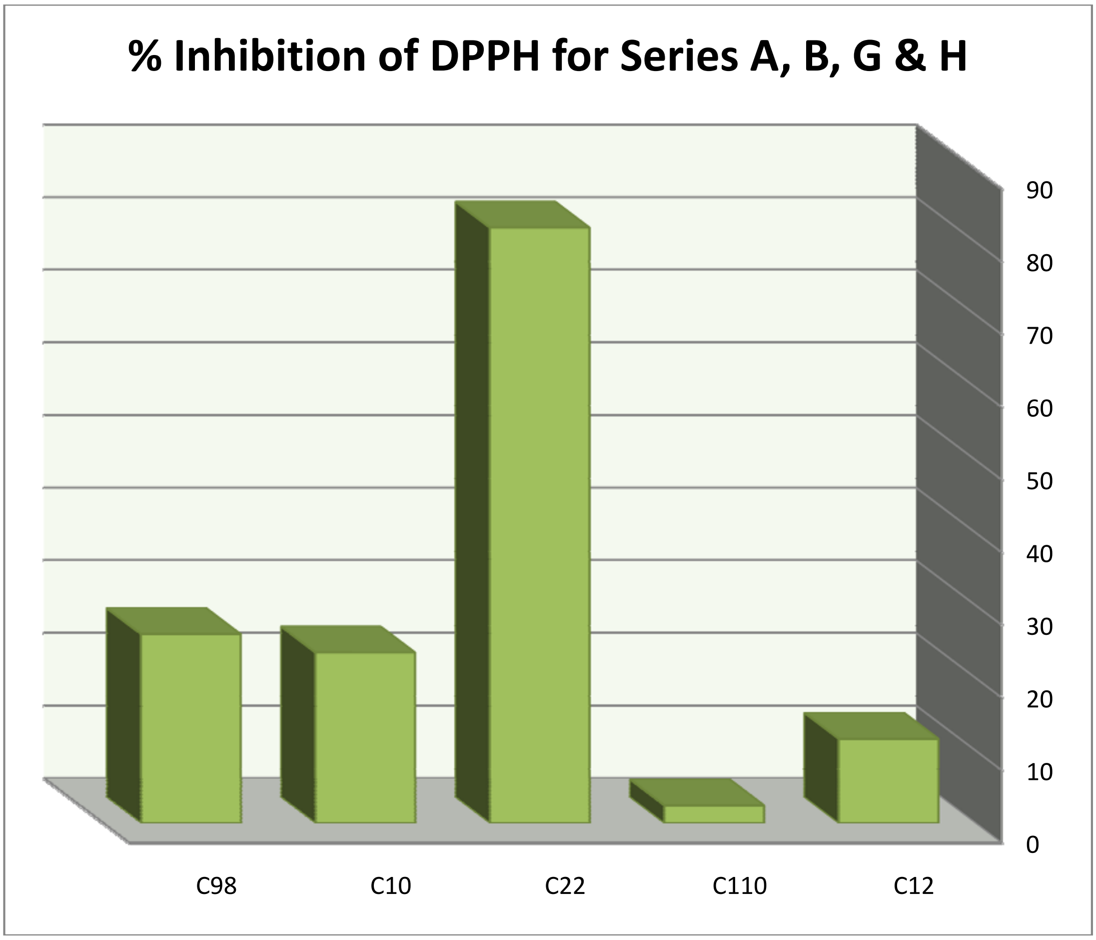


Figure 72: The antioxidant activity of Series A, B, G and H (DPPH assay

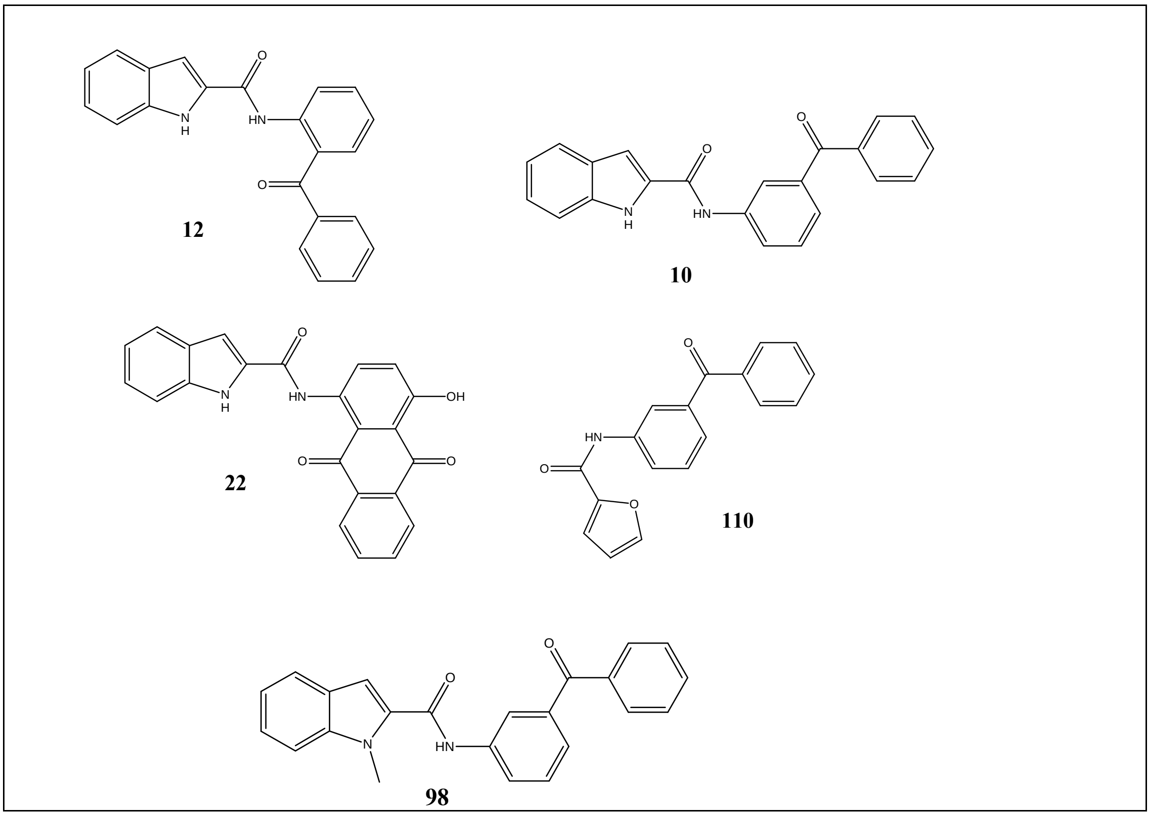


Figure 73: The Chemical structures of compounds **98, 10, 22, 110** and **12.**

As previously discussed for scavenger activity, the total number and location of hydroxyl groups on aromatic rings of synthesized compounds are of great importance in lipid peroxidation inhibition (Ochani and Mello, 2009). In this study it was demonstrated that at least one OH groups must be present in the structure in order to produce antioxidant activity.

In conclusion, it is evident that the properties of these derivatives as antioxidant and scavenger of oxygen free radicals appeared to be mediated through radical addition/by electron transfer/or by hydrogen abstraction from compounds, wherein by virtue of extended conjugation the compound has the ability to delocalize unpaired electrons (Figure 74) (Wanasundara and Shahidi, 2005).

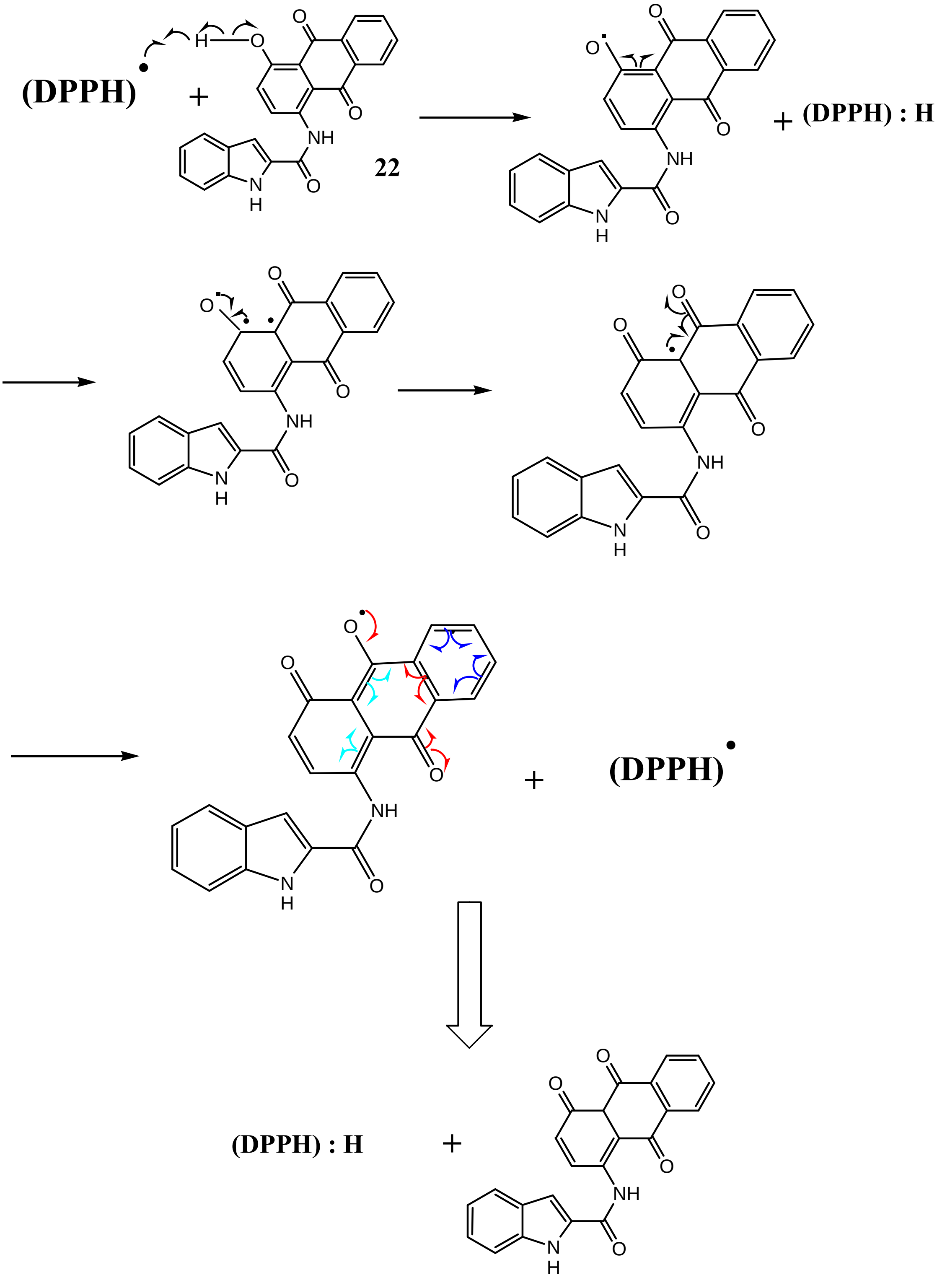


Figure 74: Proposed reaction mechanism between (DPPH) radical and **C22.**

10. Conclusion

The research described in this thesis focuses on the preparation, characterization and elucidation of the function of these synthetic compounds as lipid lowering and antioxidant agents. Aseries of novel unsubstituted, substituted indole 2-carboxamide and their isoster counterparts the pyrrole and furan 2-carboxamide have shown significant effect on lipid profile in the rats model.

The findings of the study suggest that compounds **6, 18, 24, 26, 36, 88, 90, 93, 103, 104** and **111** at 57 µM after 8 hrs of Triton injection showed excellent antidyslipidemic activities, lowering TC, TG, LDL, VLDL and increasing HDL levels, and were even better than that of the reference standard bezafibrate. The mechanism has been pointed towards agonism of PPARα.

In addition compounds **22, 57, 30, 55** and **59** showed excellent percent inhibition of DPPH activity and were the most effective DPPH radical scavengers. The ability of these compounds to scavange DPPH radicals suggests that they are electron donor and can react with free radicals to convert them to more stable products and terminate radical chain reactions.

Further studies are needed to elucidate whether they could be useful in the management of human diseases resulting from oxidative stress. Therefore, those compounds that exhibited both antioxidant and antihyperlipidemic effect need to be investigated more, because they could be a promising candidate as a drug with dual effect. Compound **22** exhibited interesting most promising activity in both lipid lowering and antioxidant activities.

11. Future work

1. Modification of the procedures to get better yield.

2. Modify the most active compounds to get better bioactivity profile.

3. Investigate the mechanism of action of the active hits.

4. Toxicological studies.

5. Selective *in-vitro* and *in-vivo* studies on PPARα.

6. Further studies on drug metabolism to assess the biological activity.

7. Co-crystallization on LPL enzyme.

8. Docking of the active hits in the PPARα ligand-binding domain.

9**.** Use different hyperlipidemic model to test the active hits, like for example testing them on rats fed on diet rich on cholesterol to see their effect on cholesterol.

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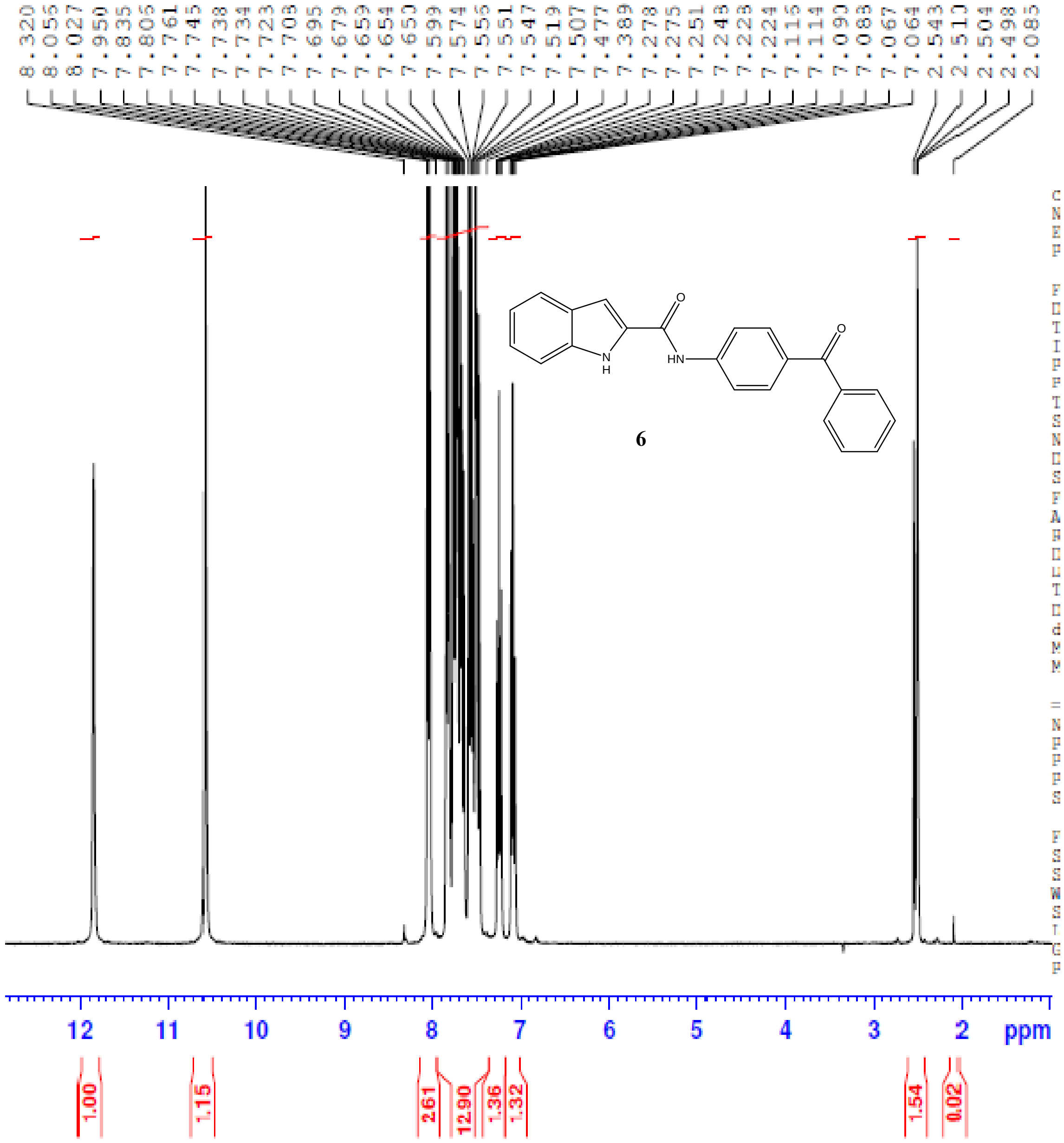
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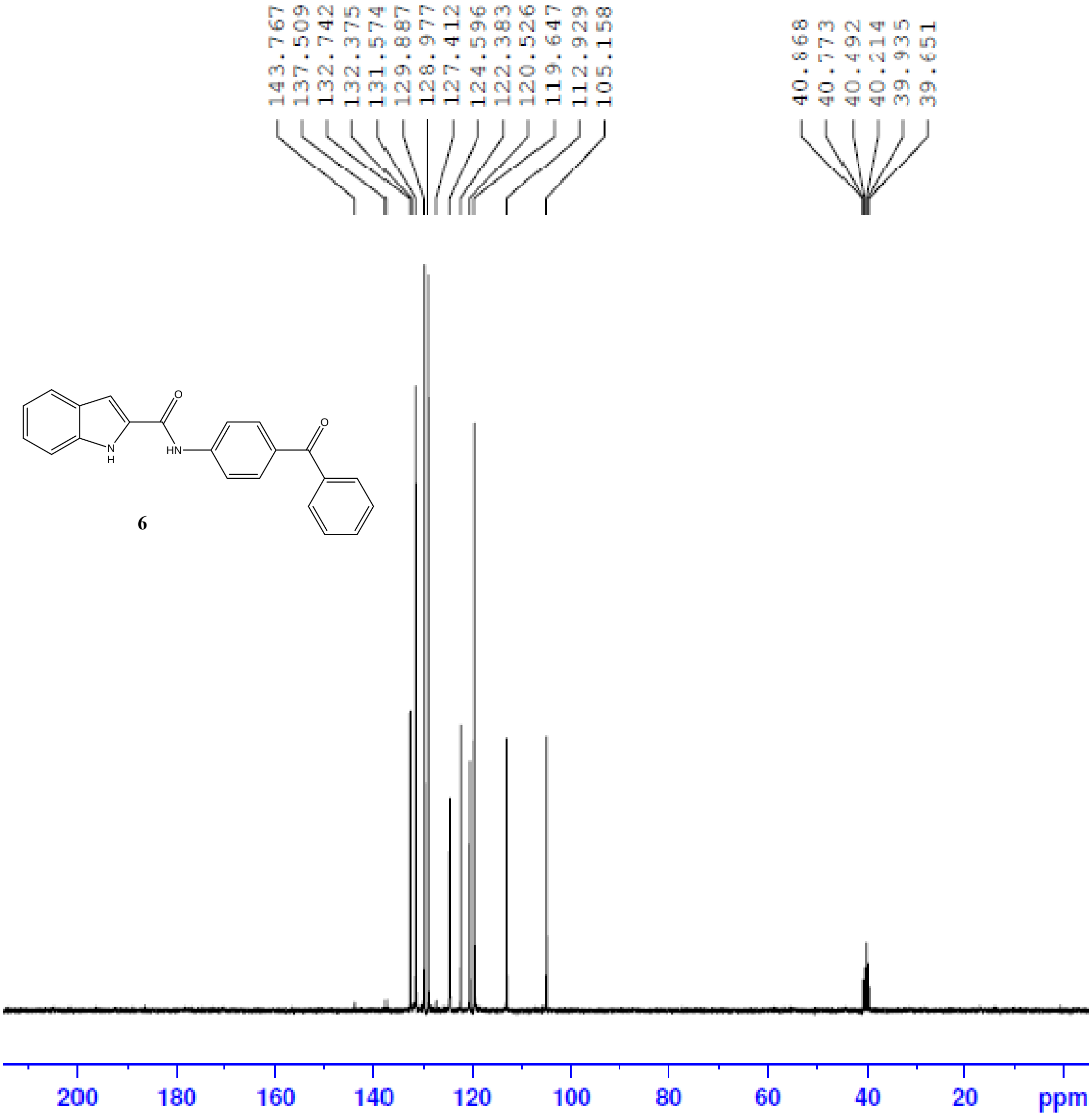
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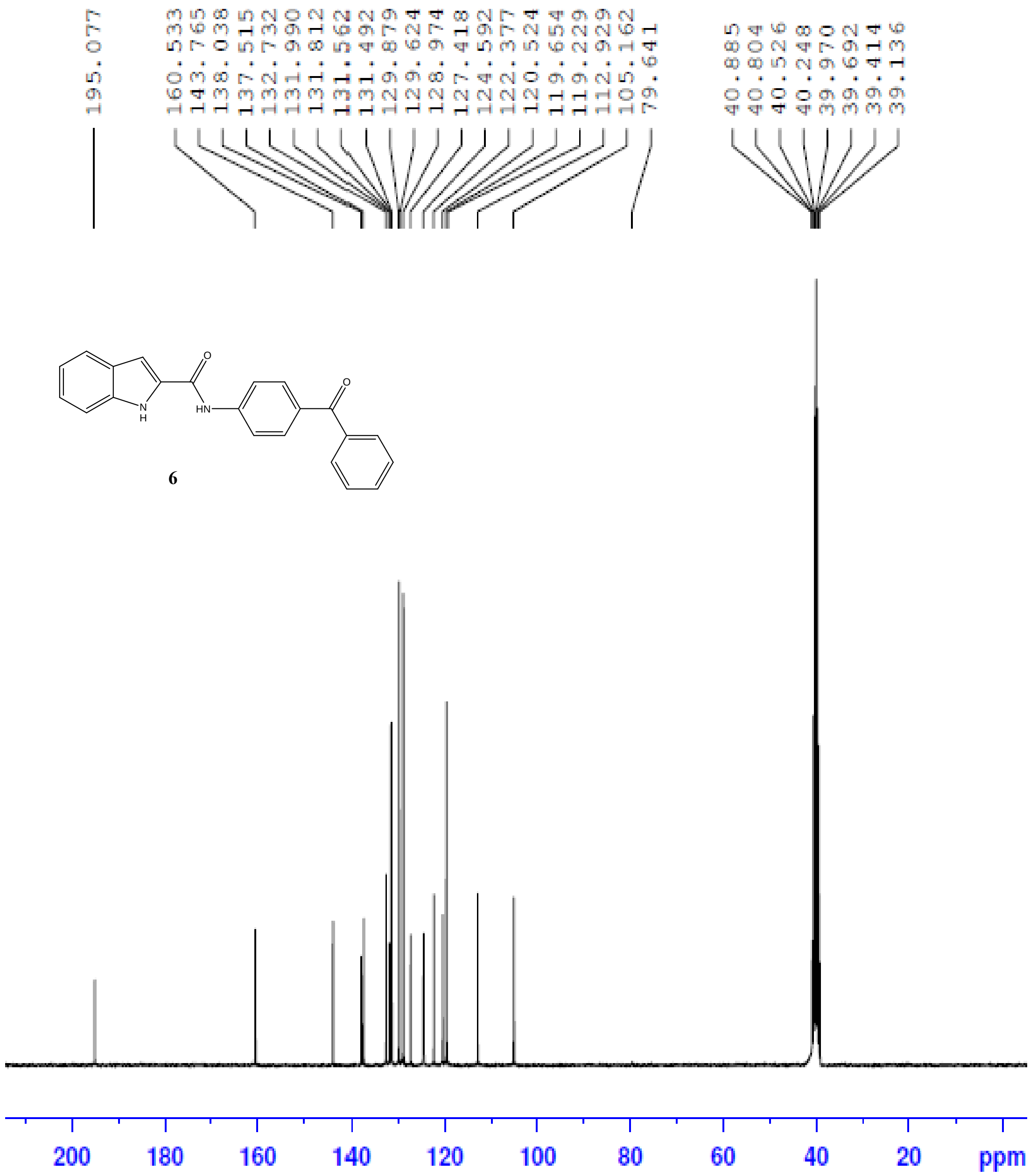
Appendix I


8.320
8.055
8.027
7.950
7.835
7.805
7.761
7.745
7.738
7.734
7.723
7.703
7.695
7.679
7.659
7.654
7.650
7.599
7.574
7.555
7.551
7.547
7.519
7.507
7.477
7.389
7.278
7.275
7.251
7.248
7.223
7.224
7.115
7.114
7.090
7.083
7.067
7.064
2.543
2.510
2.504
2.498
2.085
O
O
N
H
HN
6
12
11
10
9
8
7
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1.36
1.32
1.54
0.02

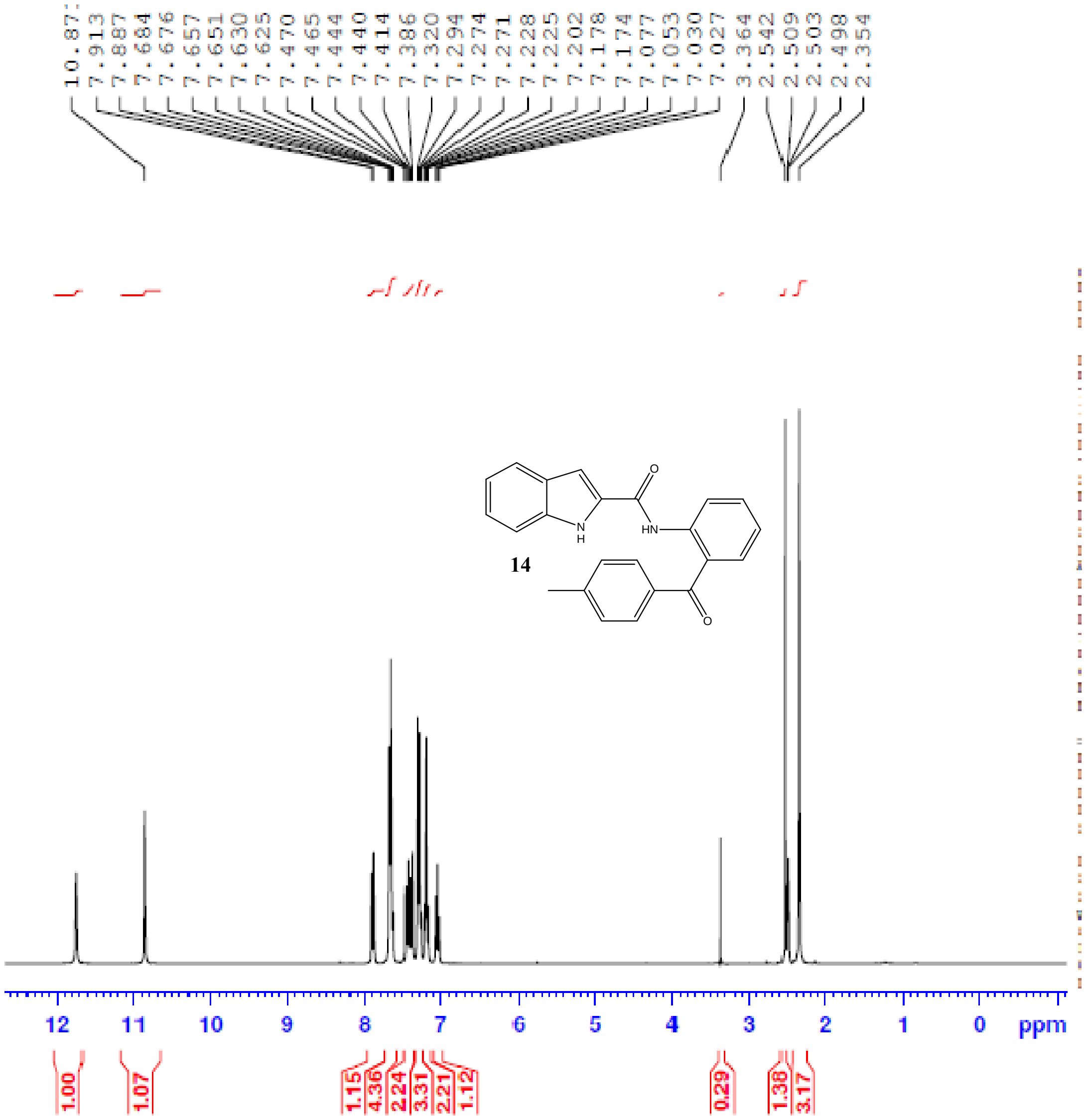
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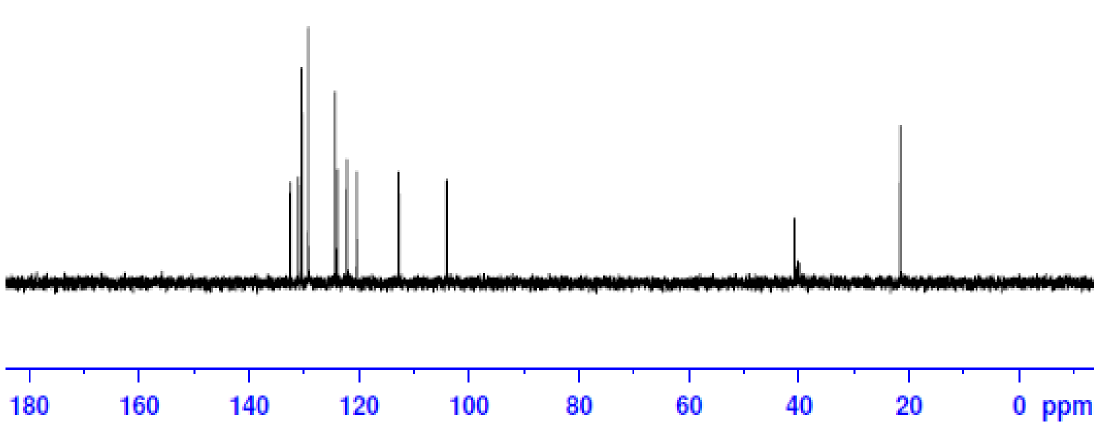
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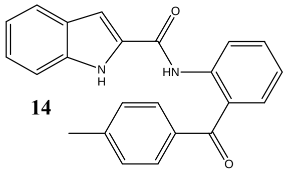


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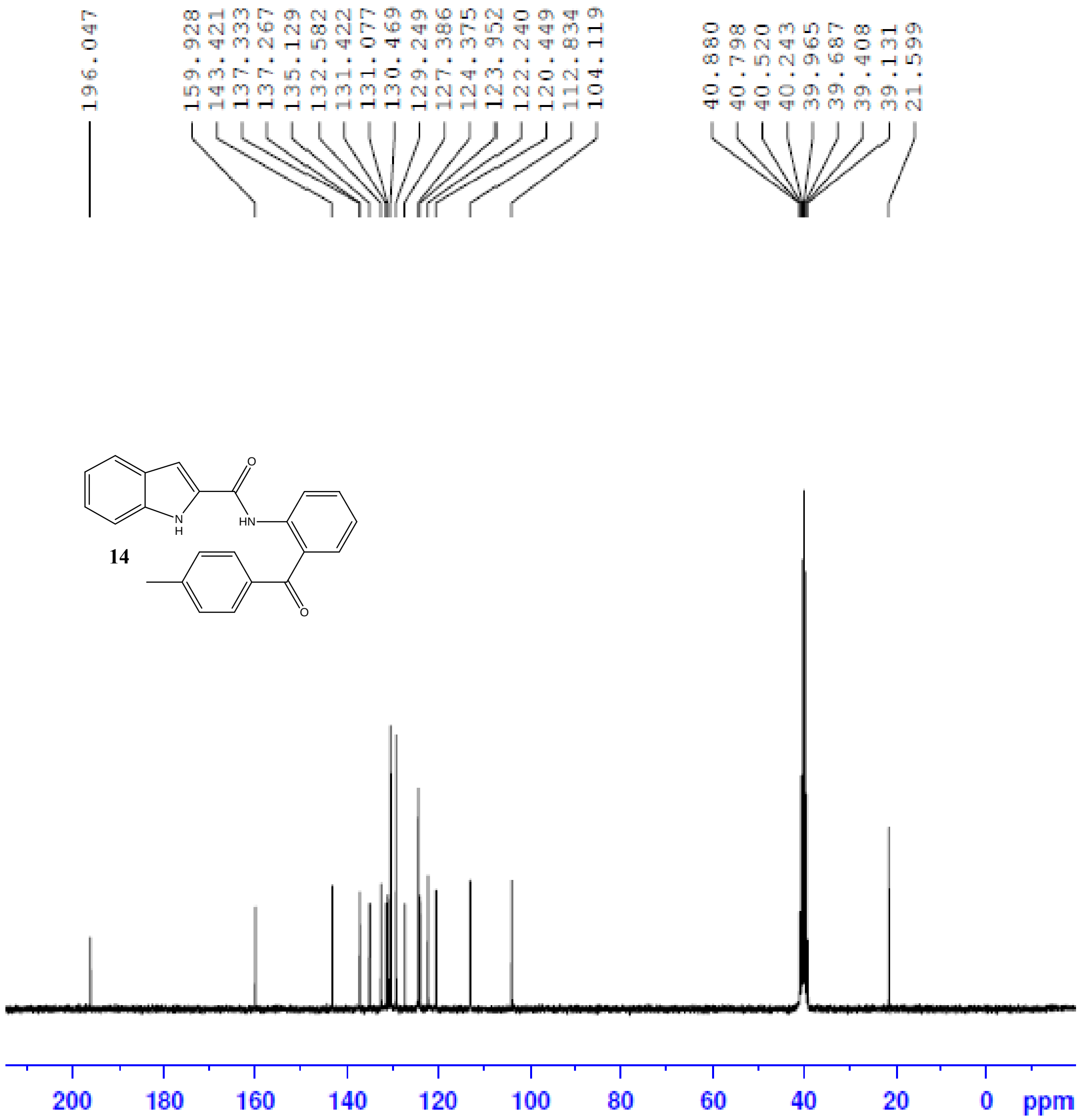
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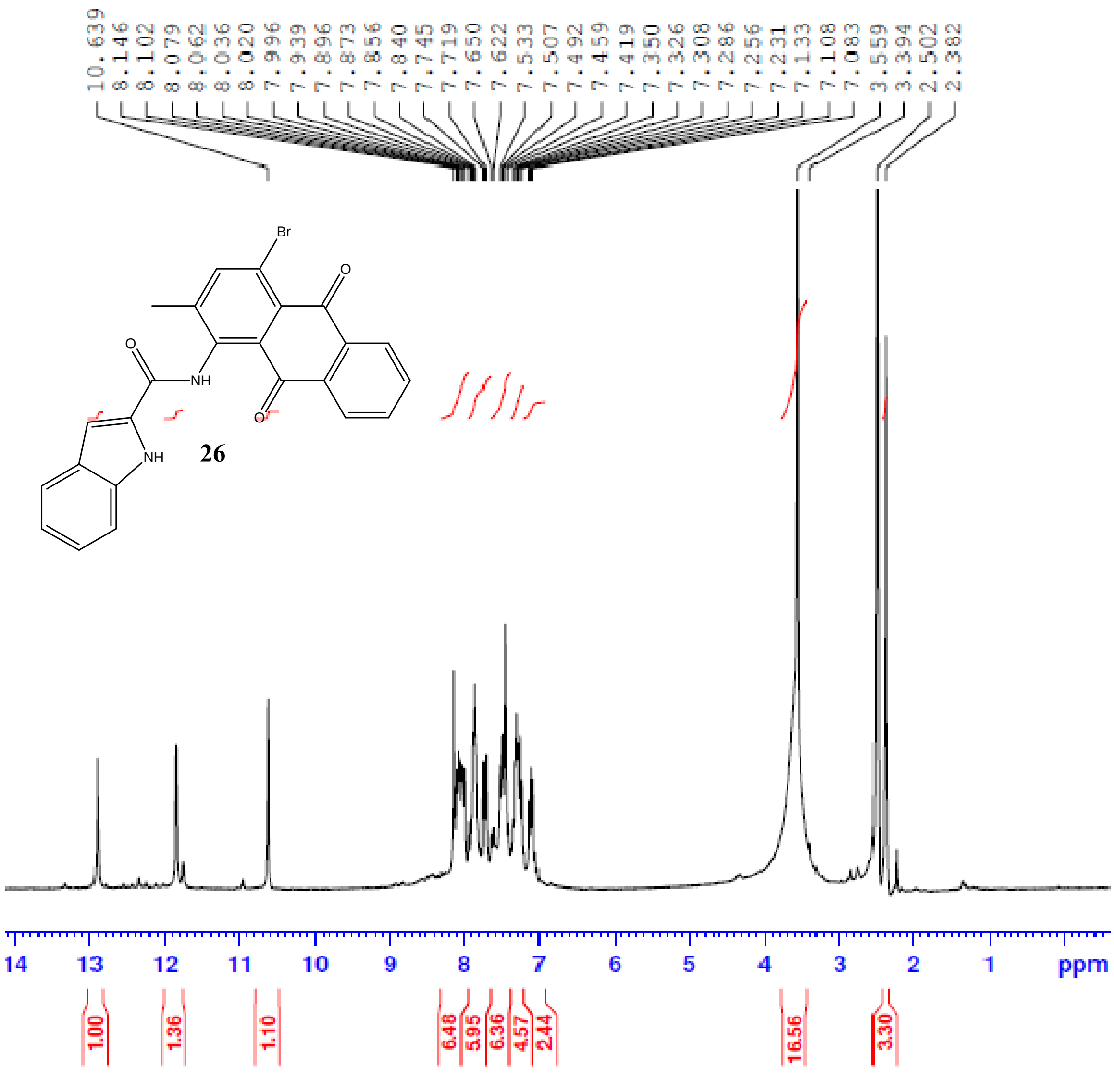


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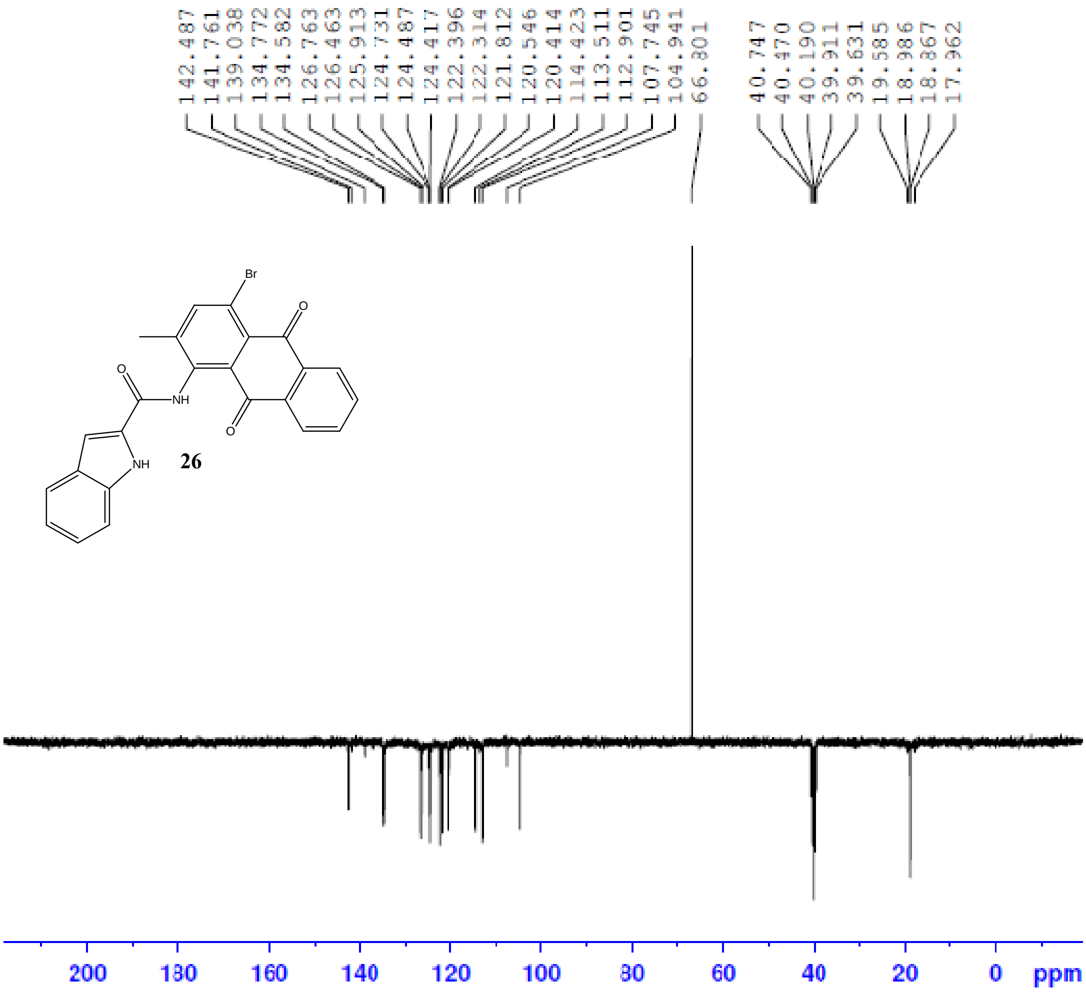
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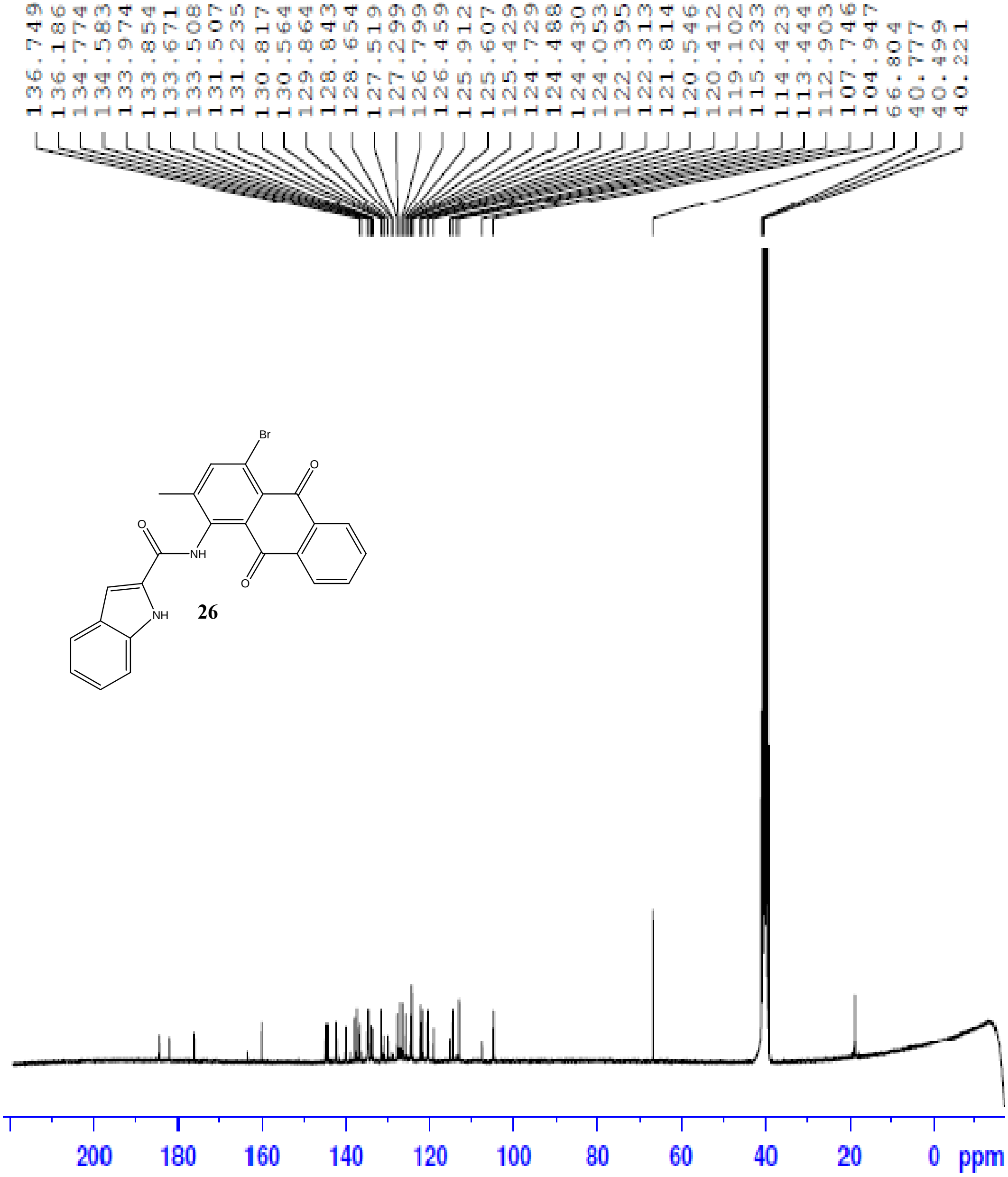
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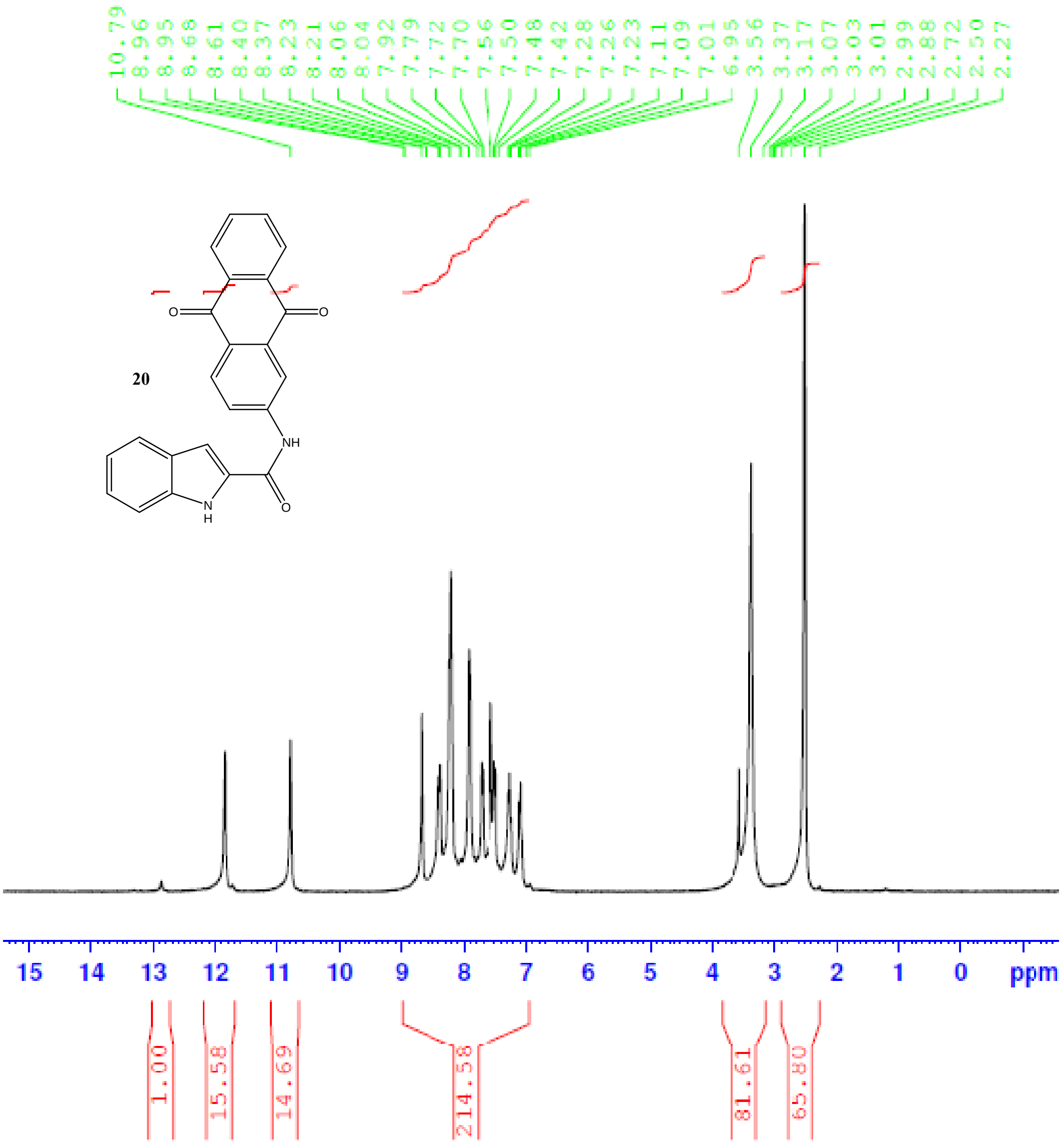
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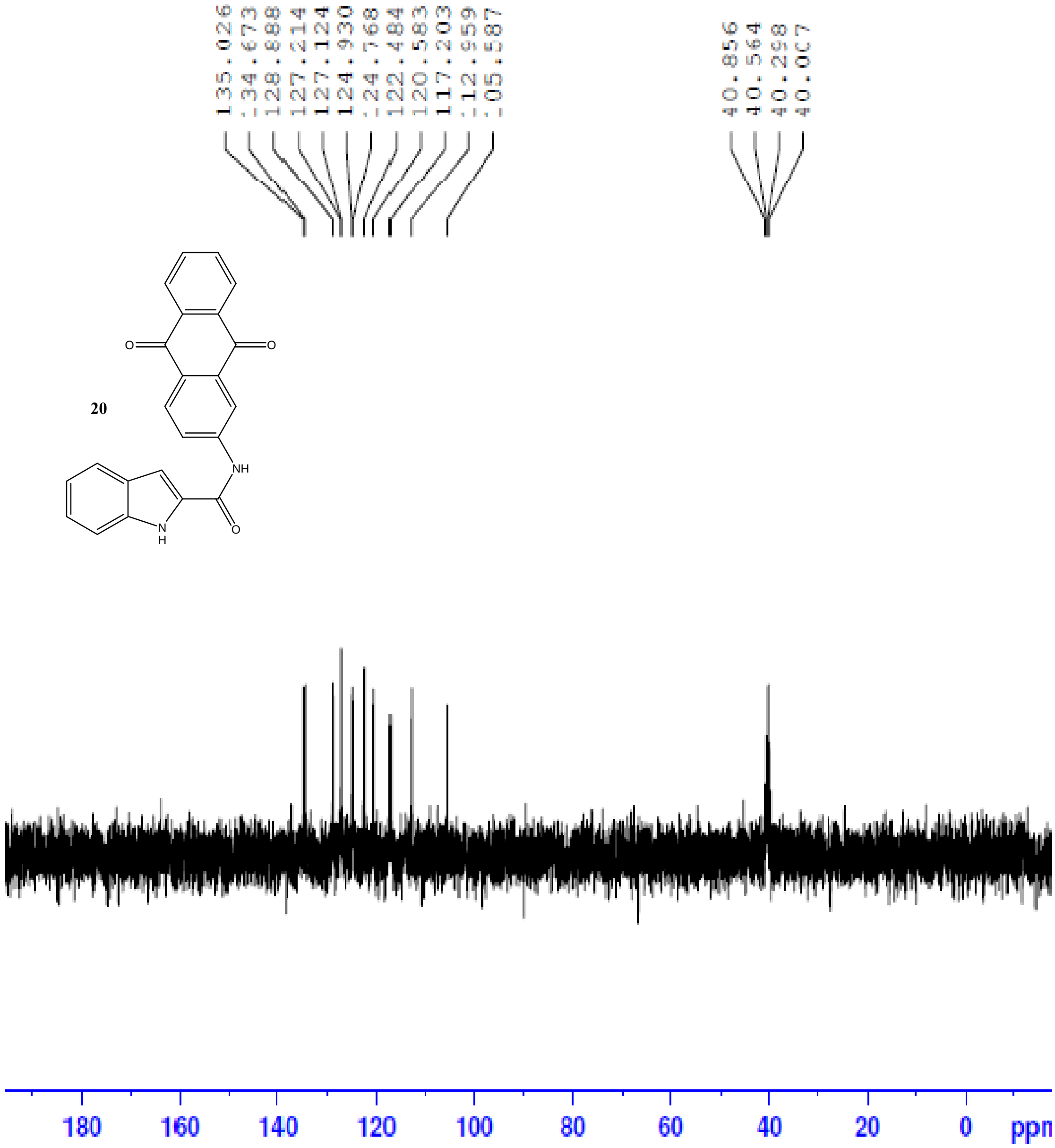
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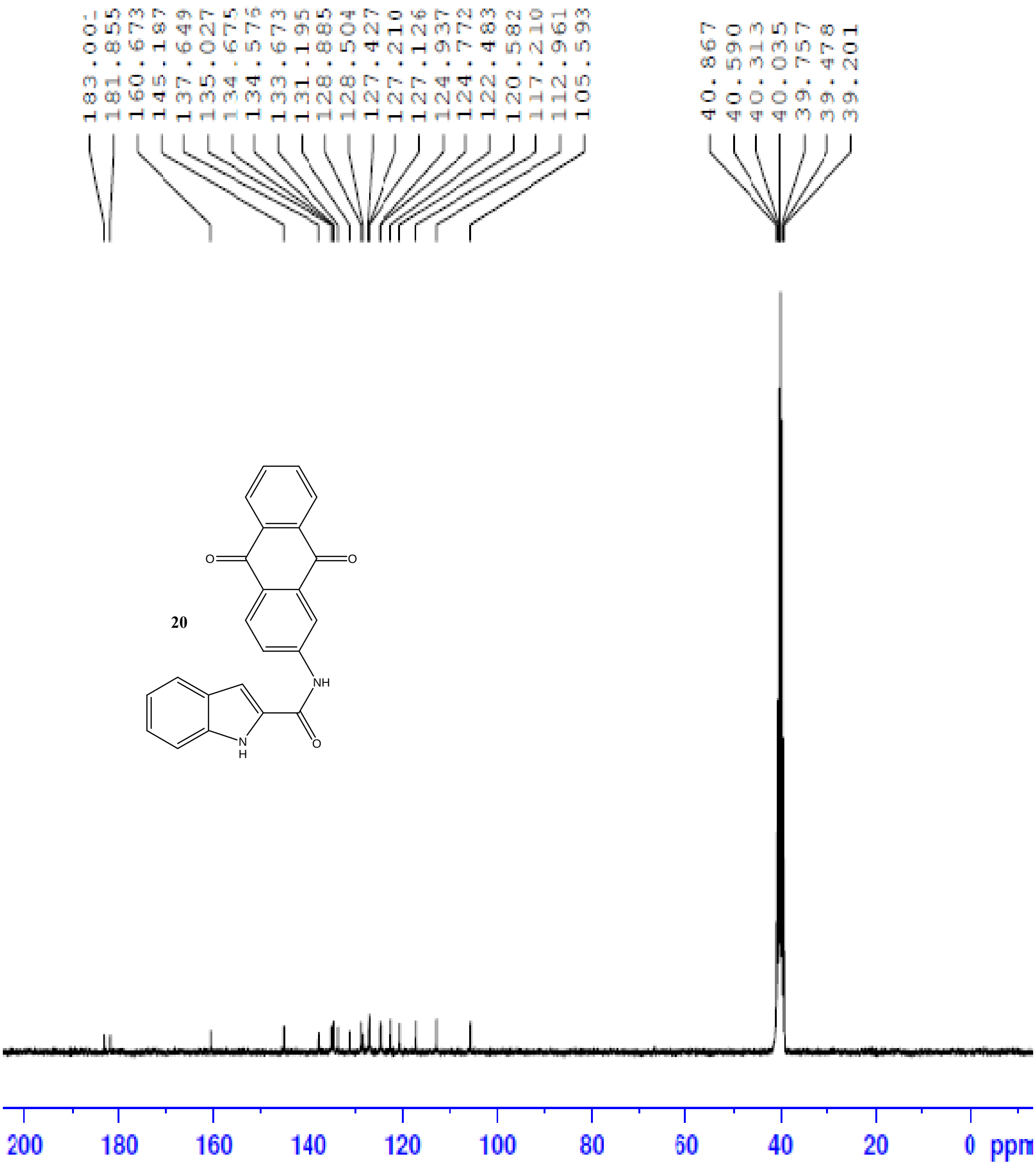
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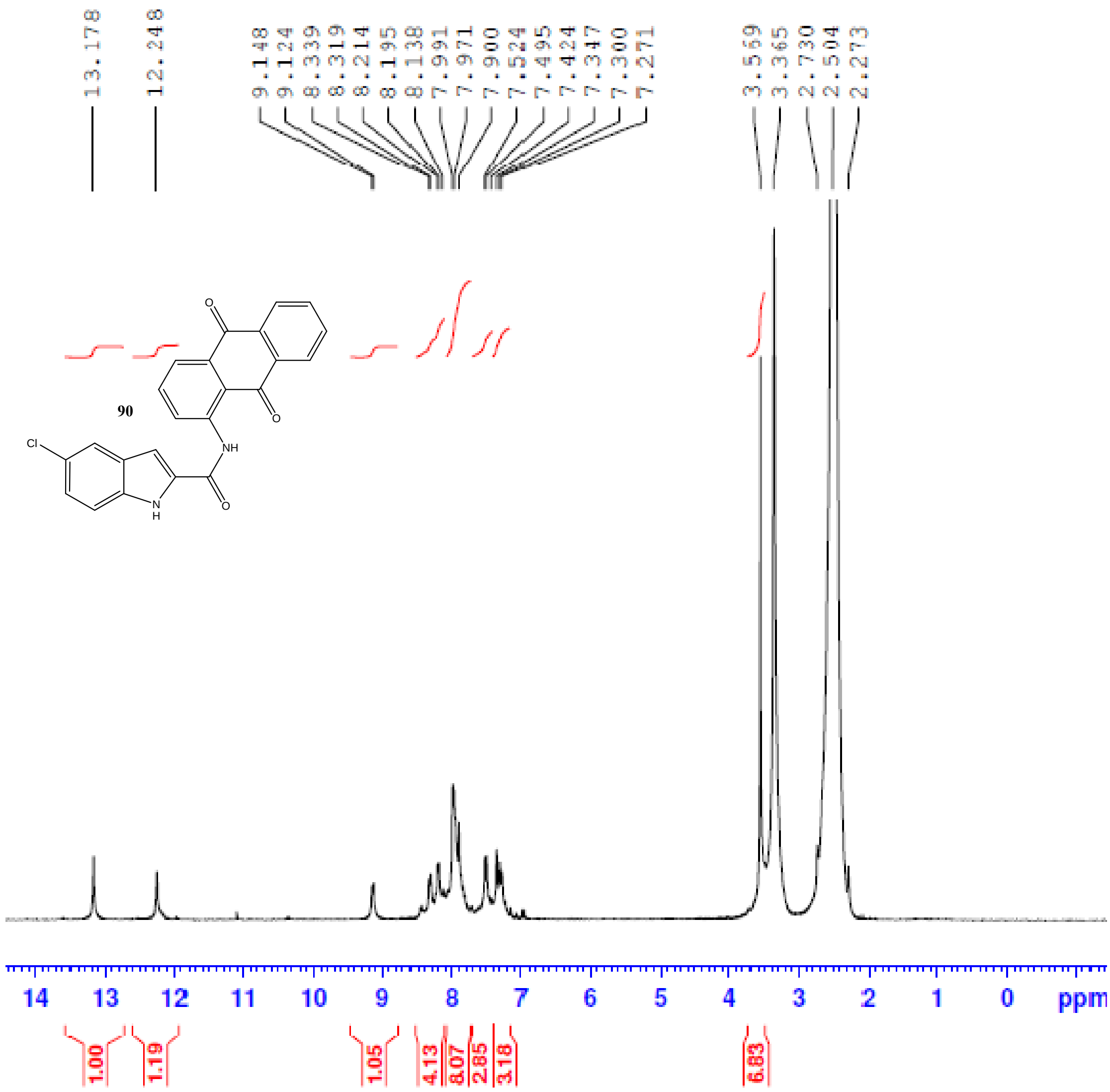
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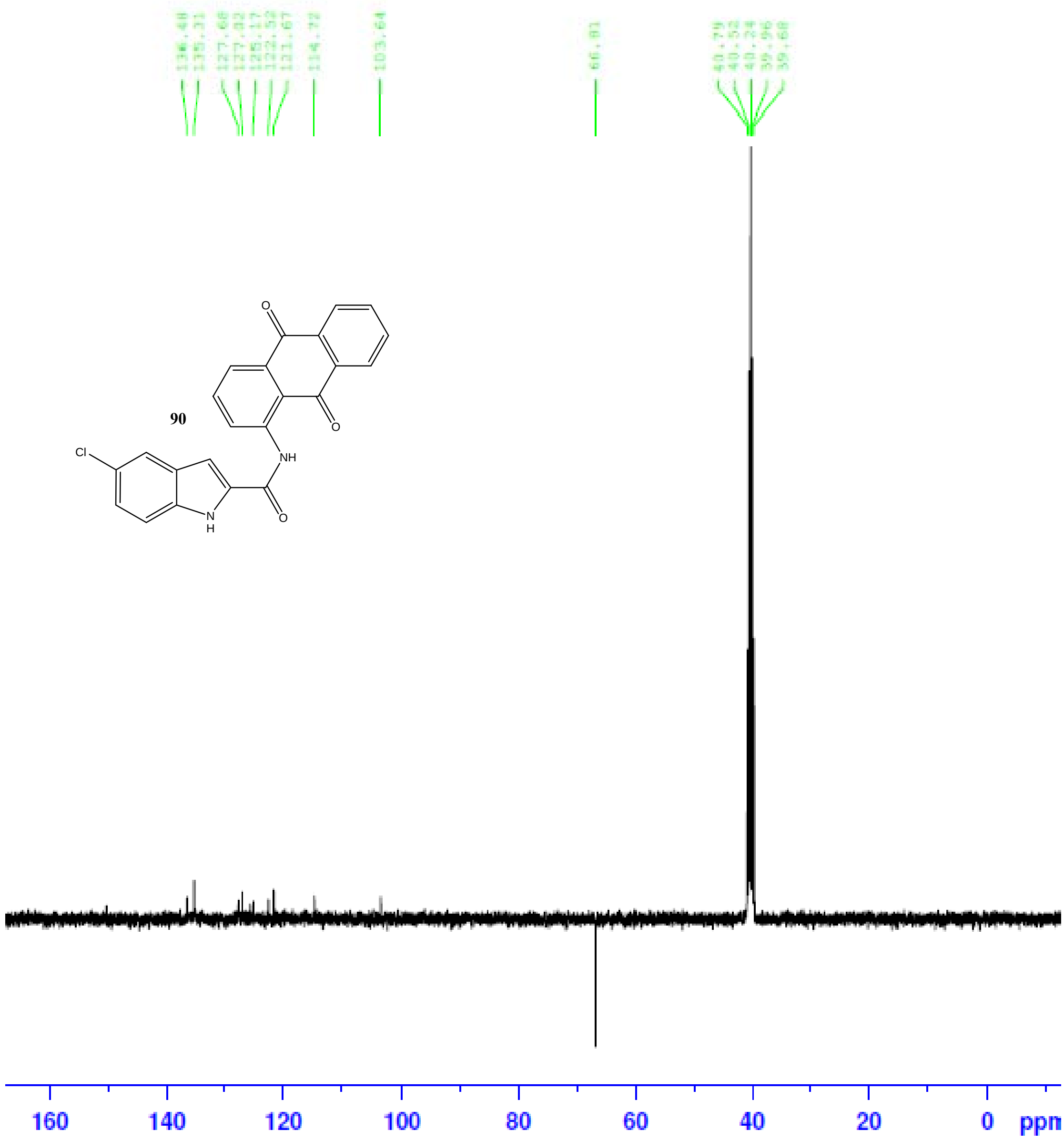
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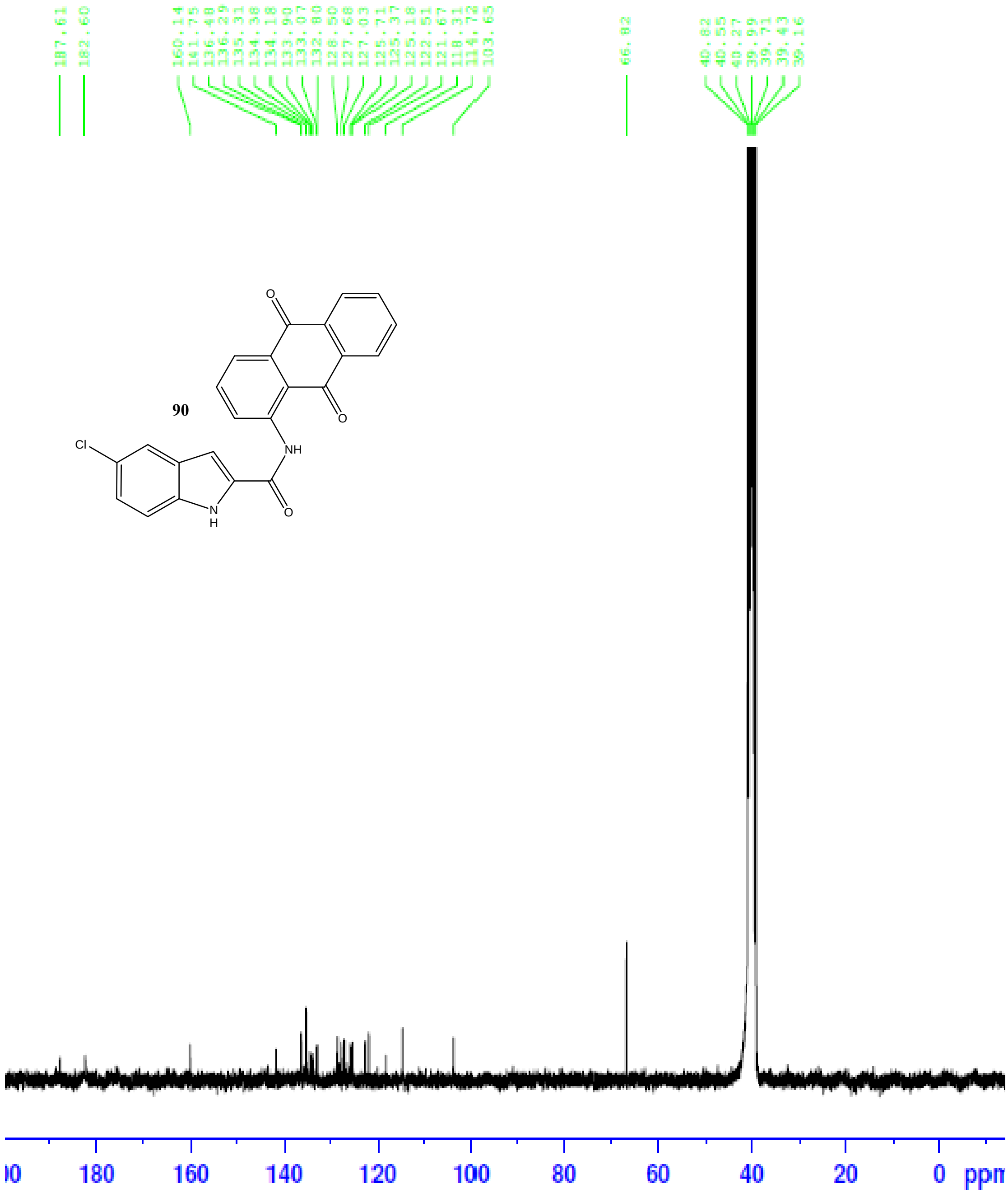
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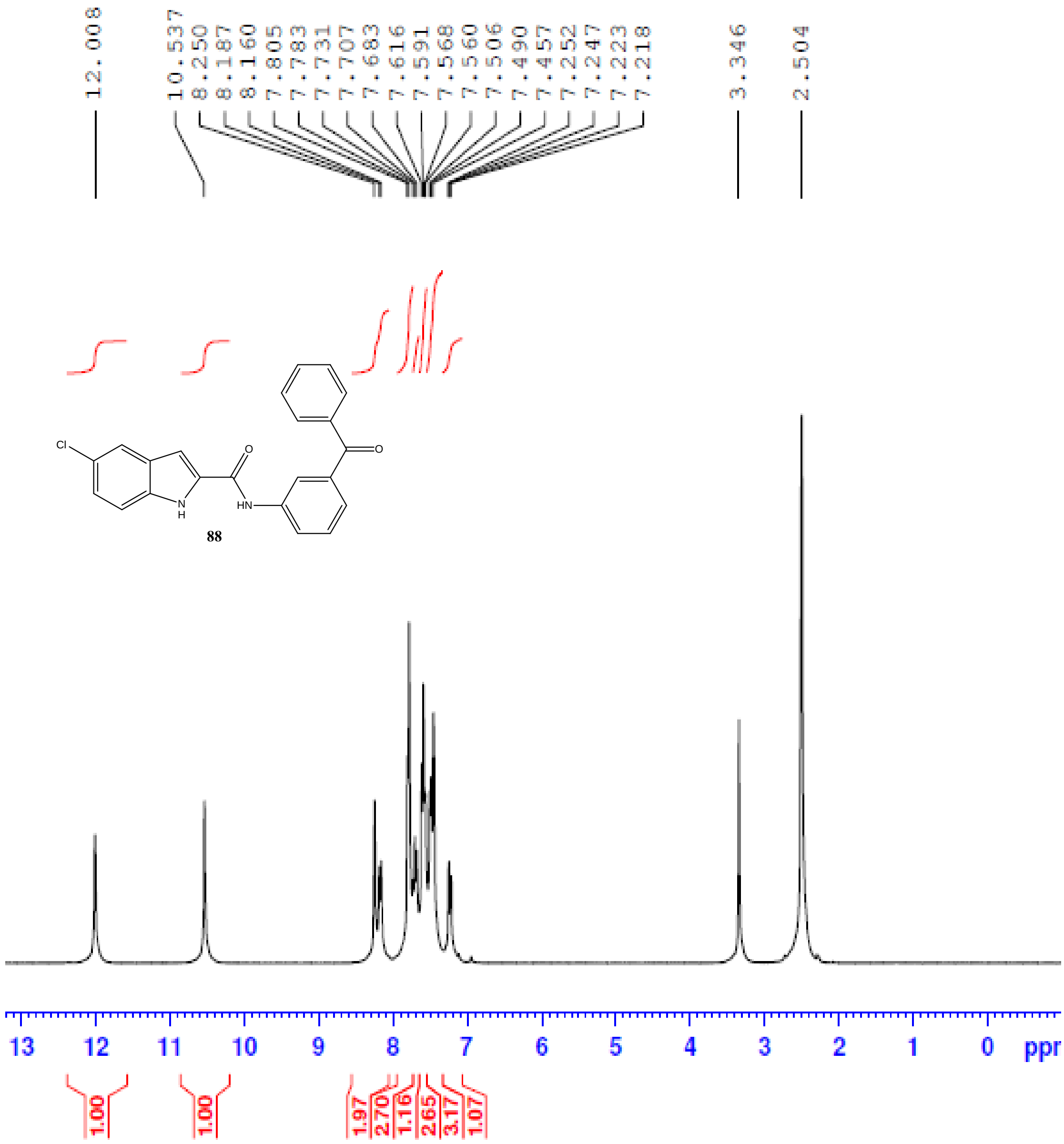
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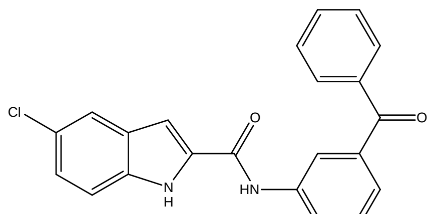
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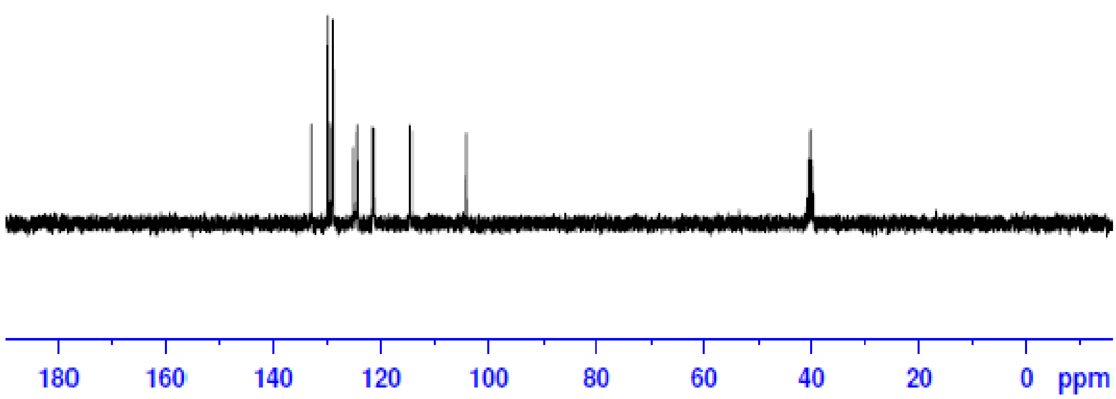
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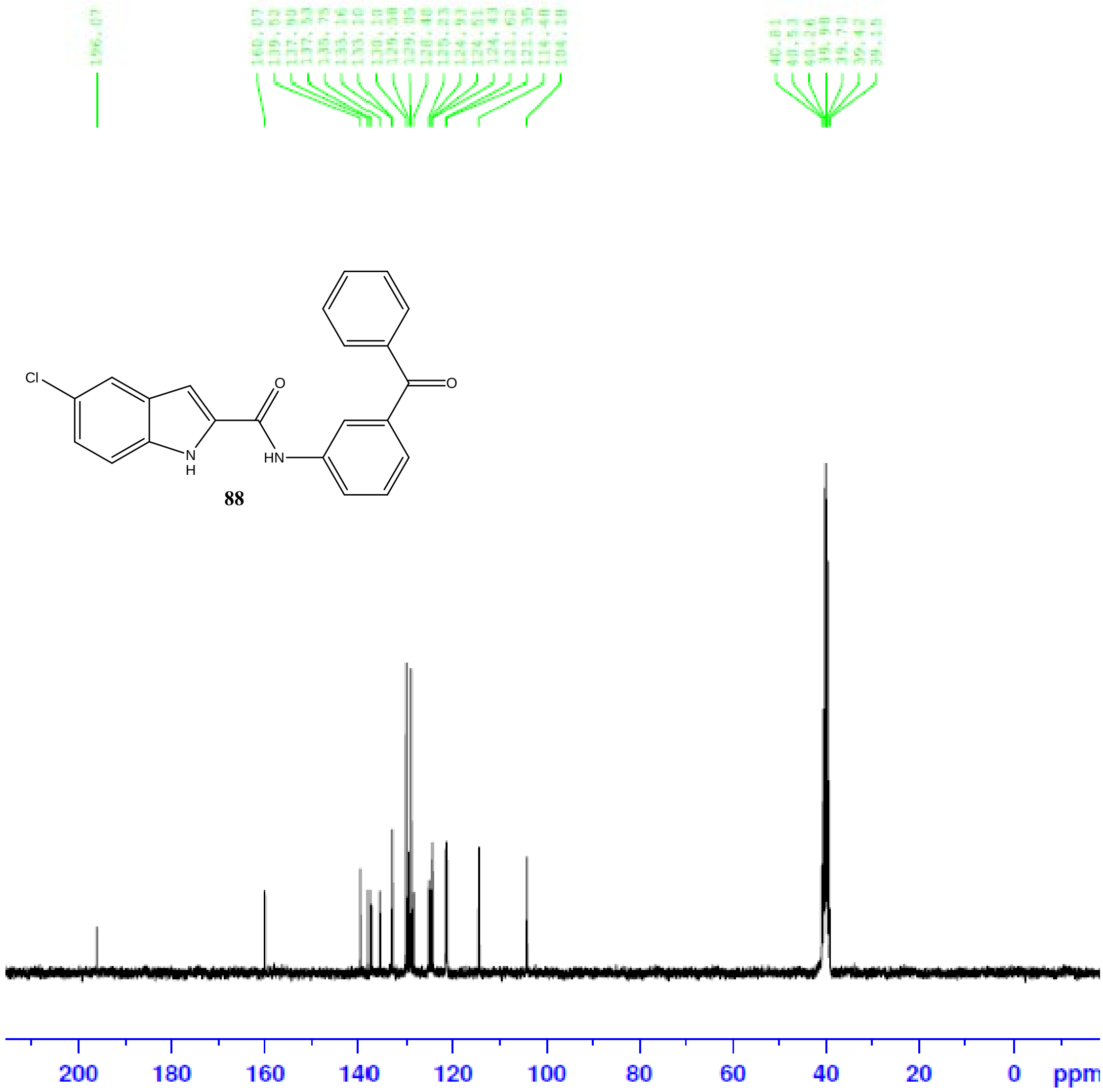
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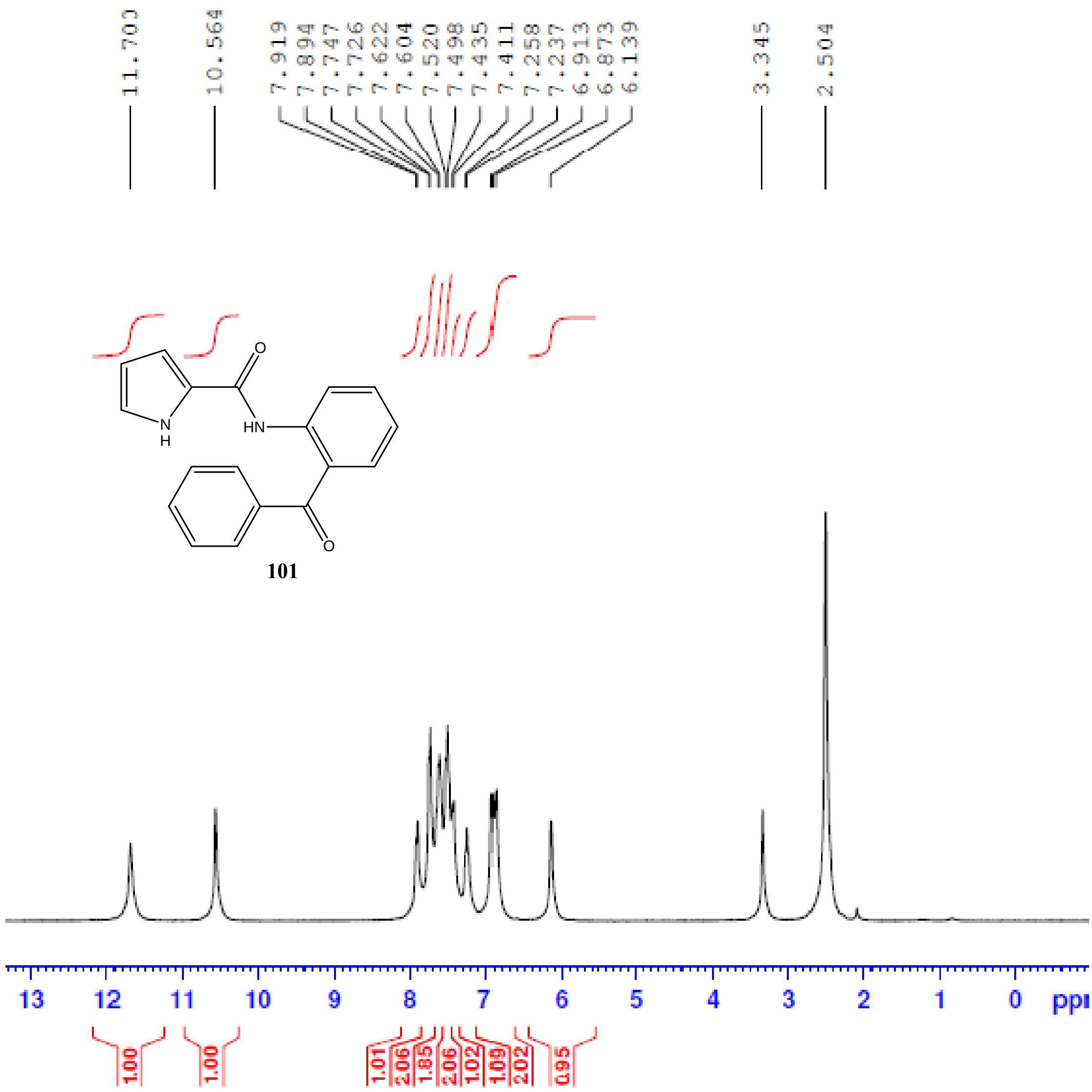
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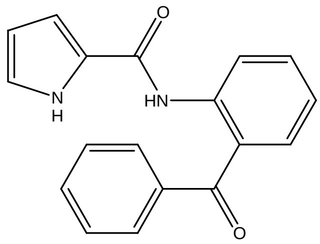
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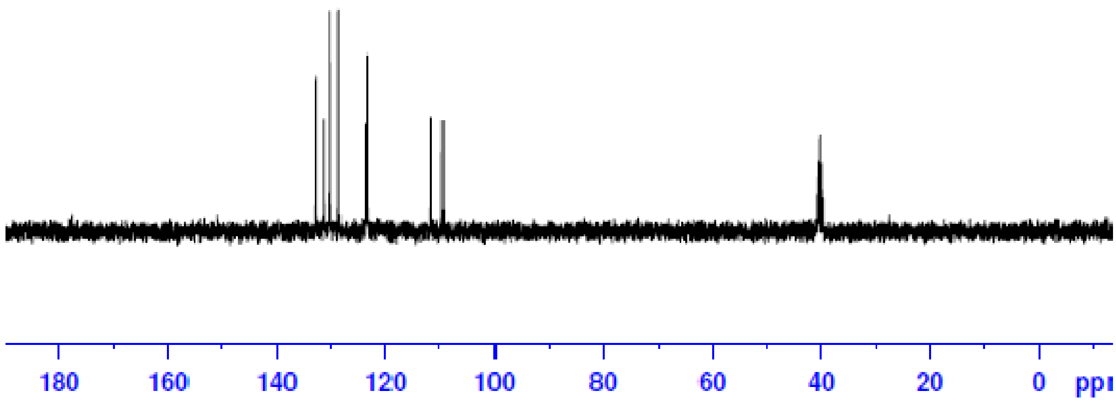
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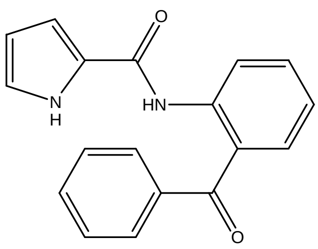
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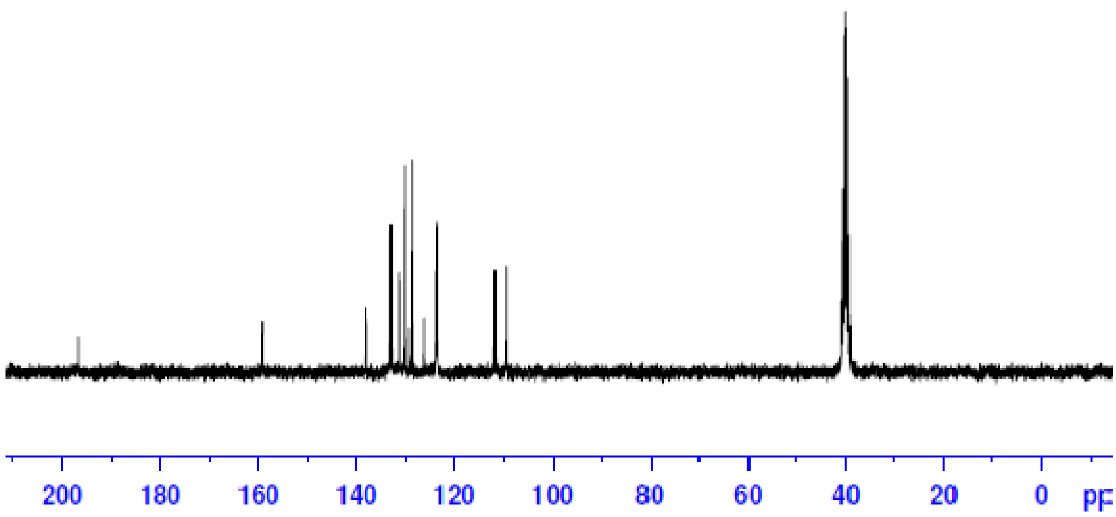
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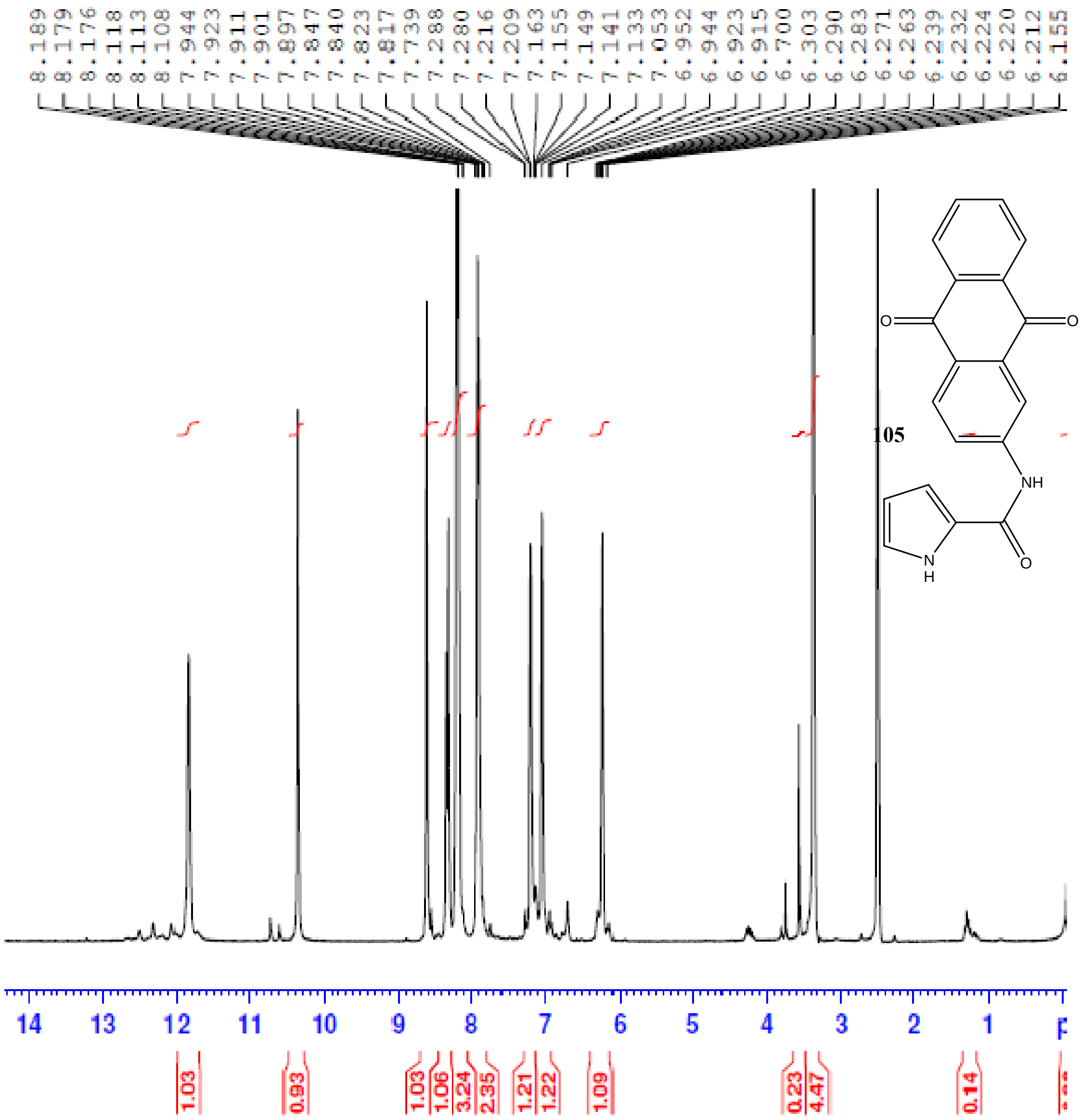
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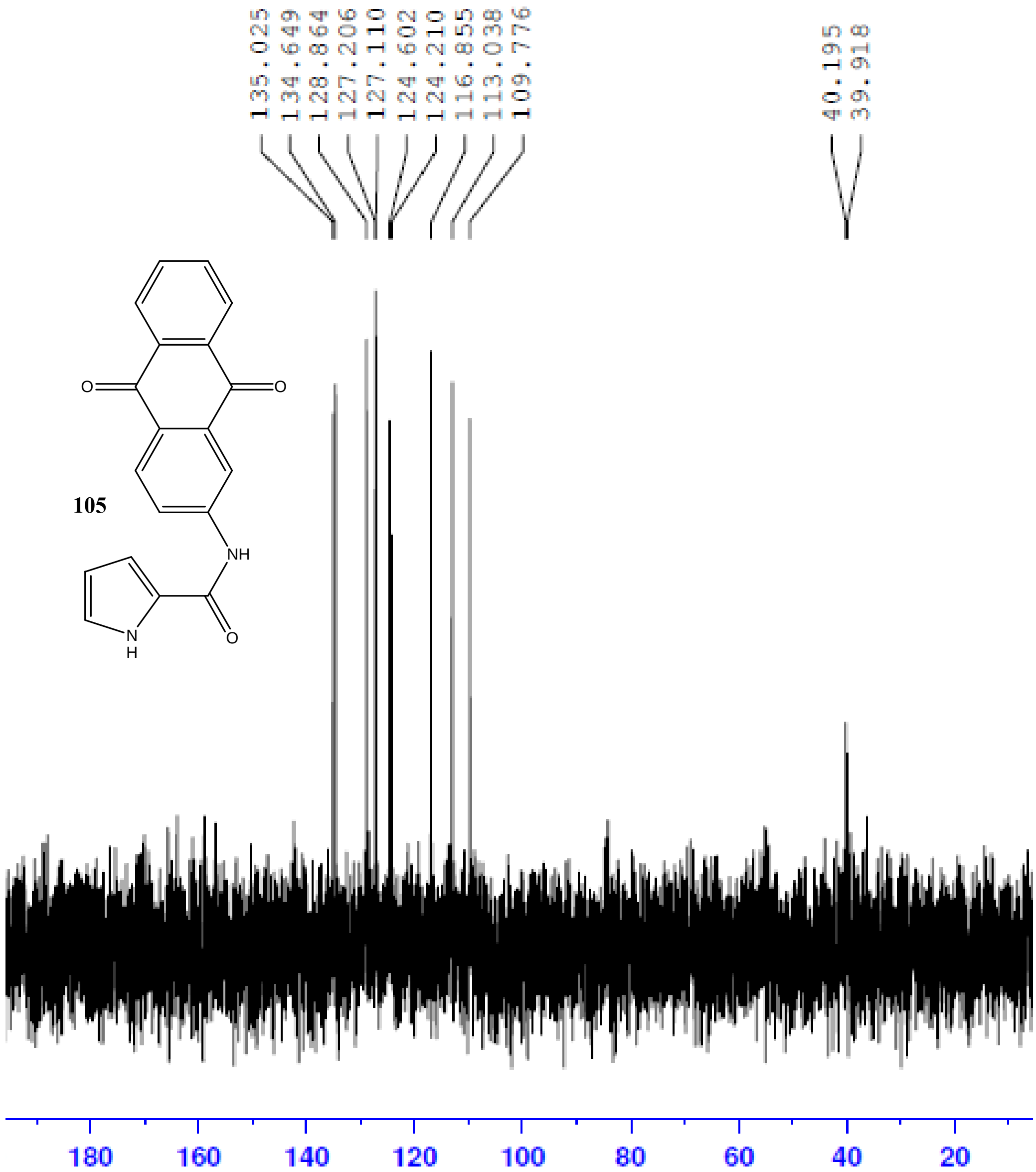
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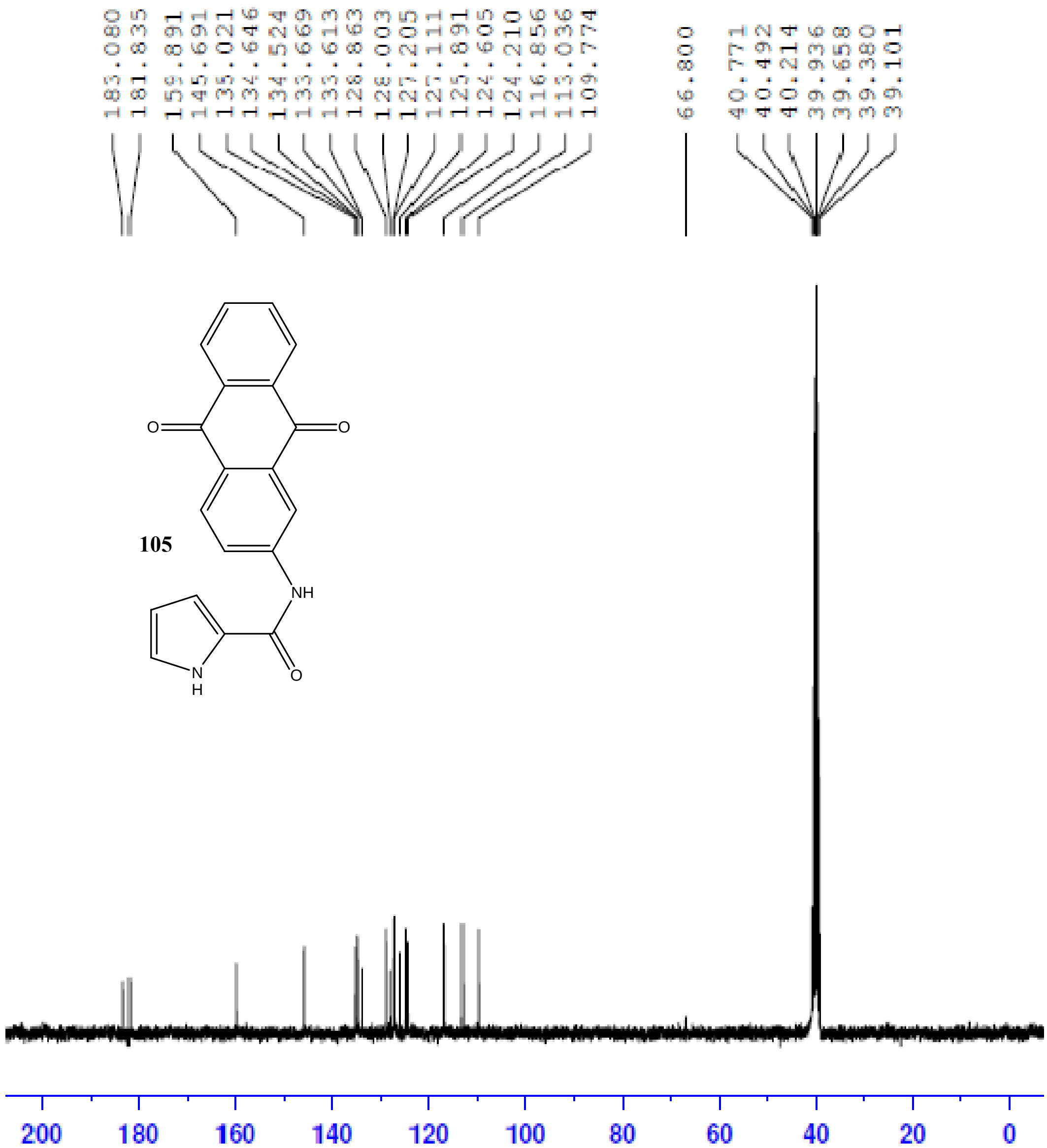
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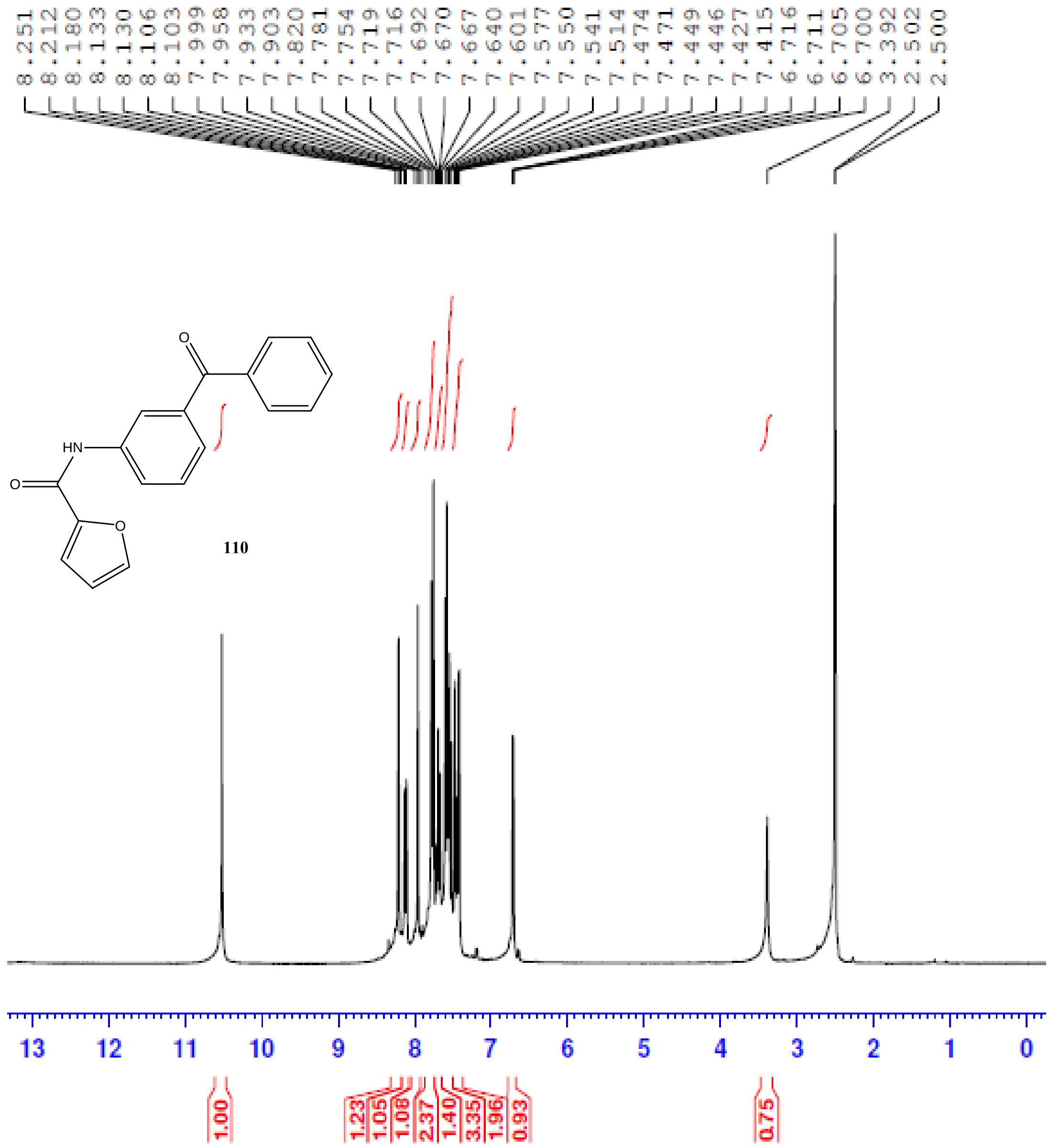
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(Dept 135 for C105)

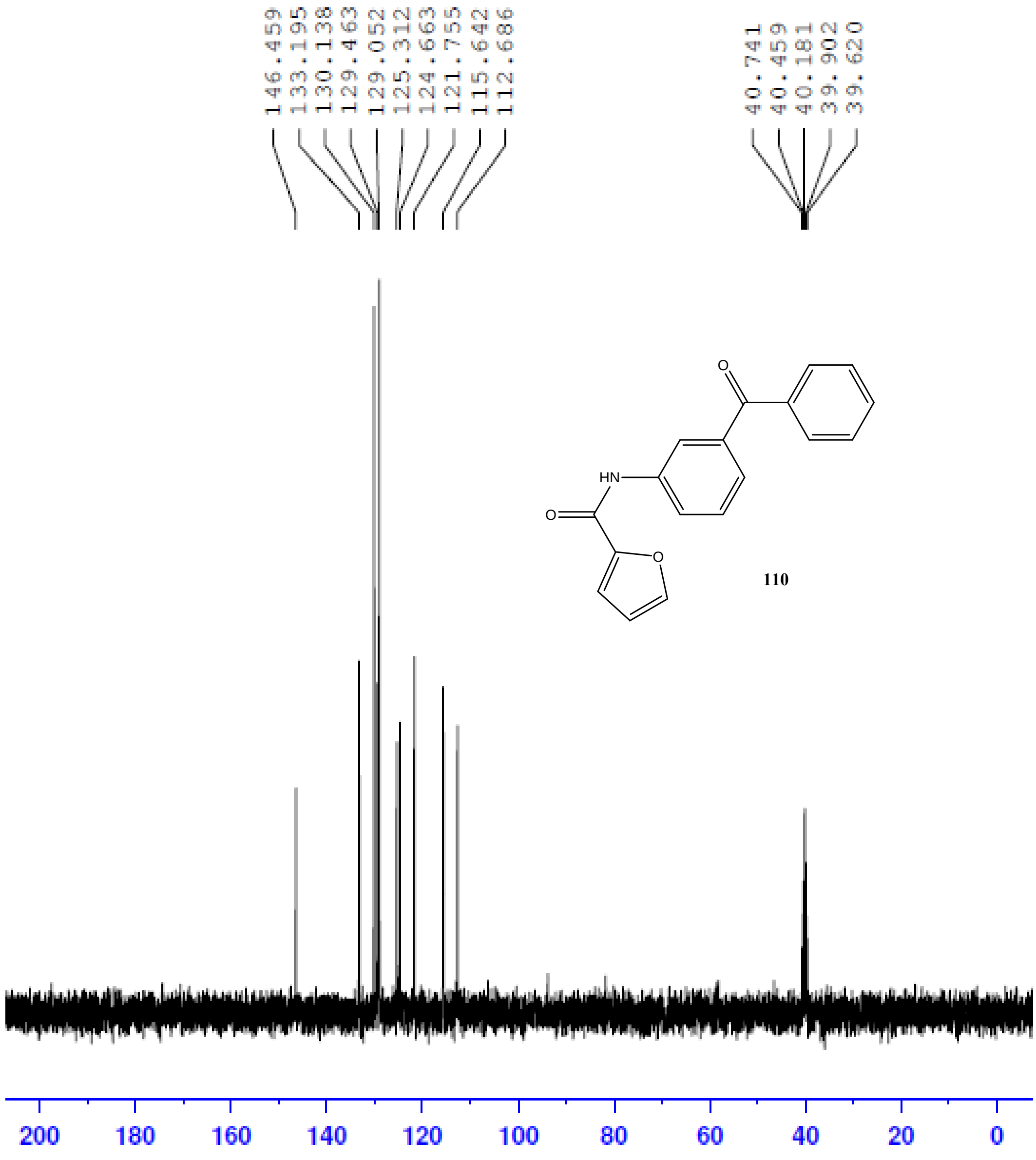


(^{13}C-NMR for C105)

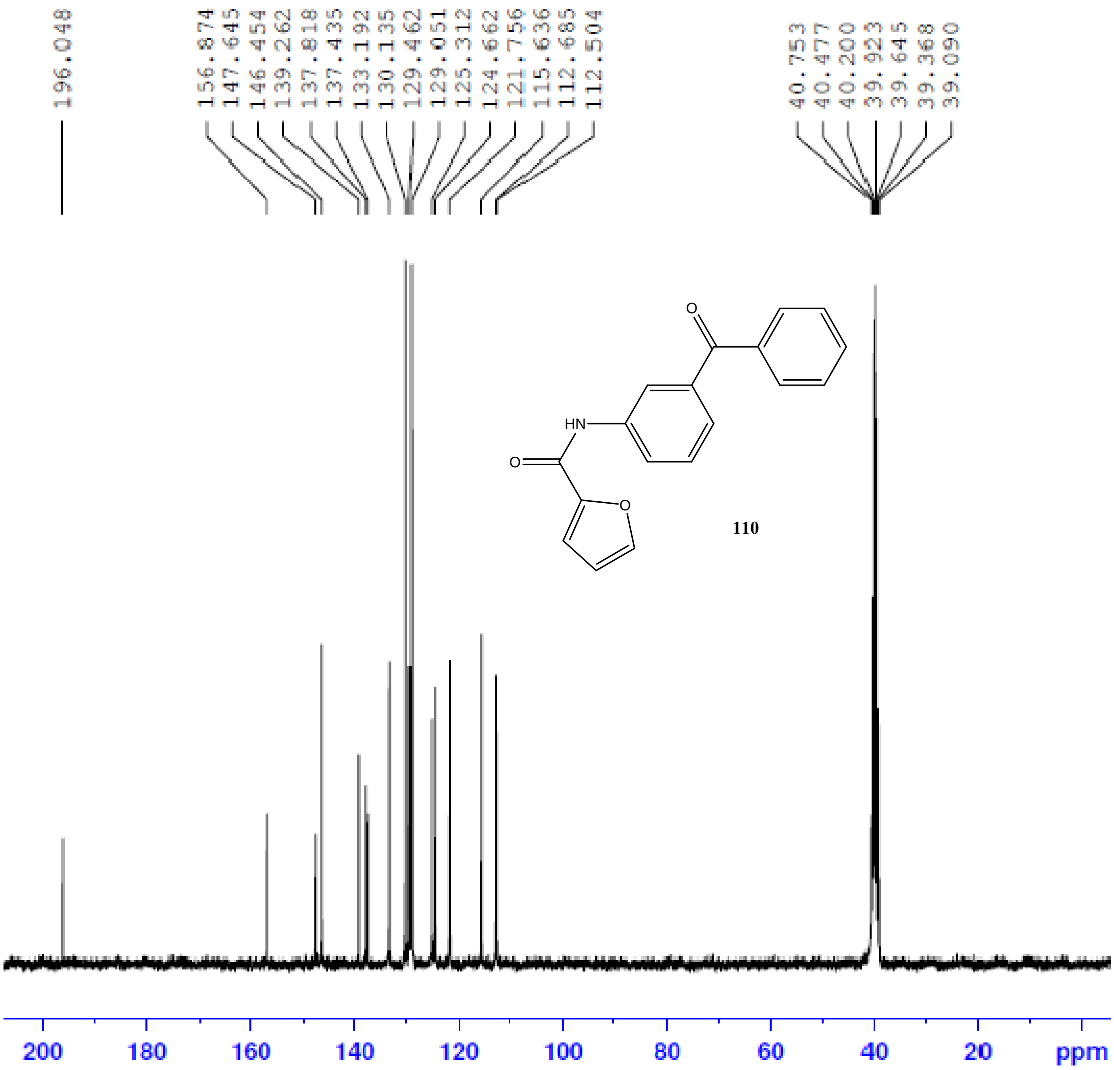


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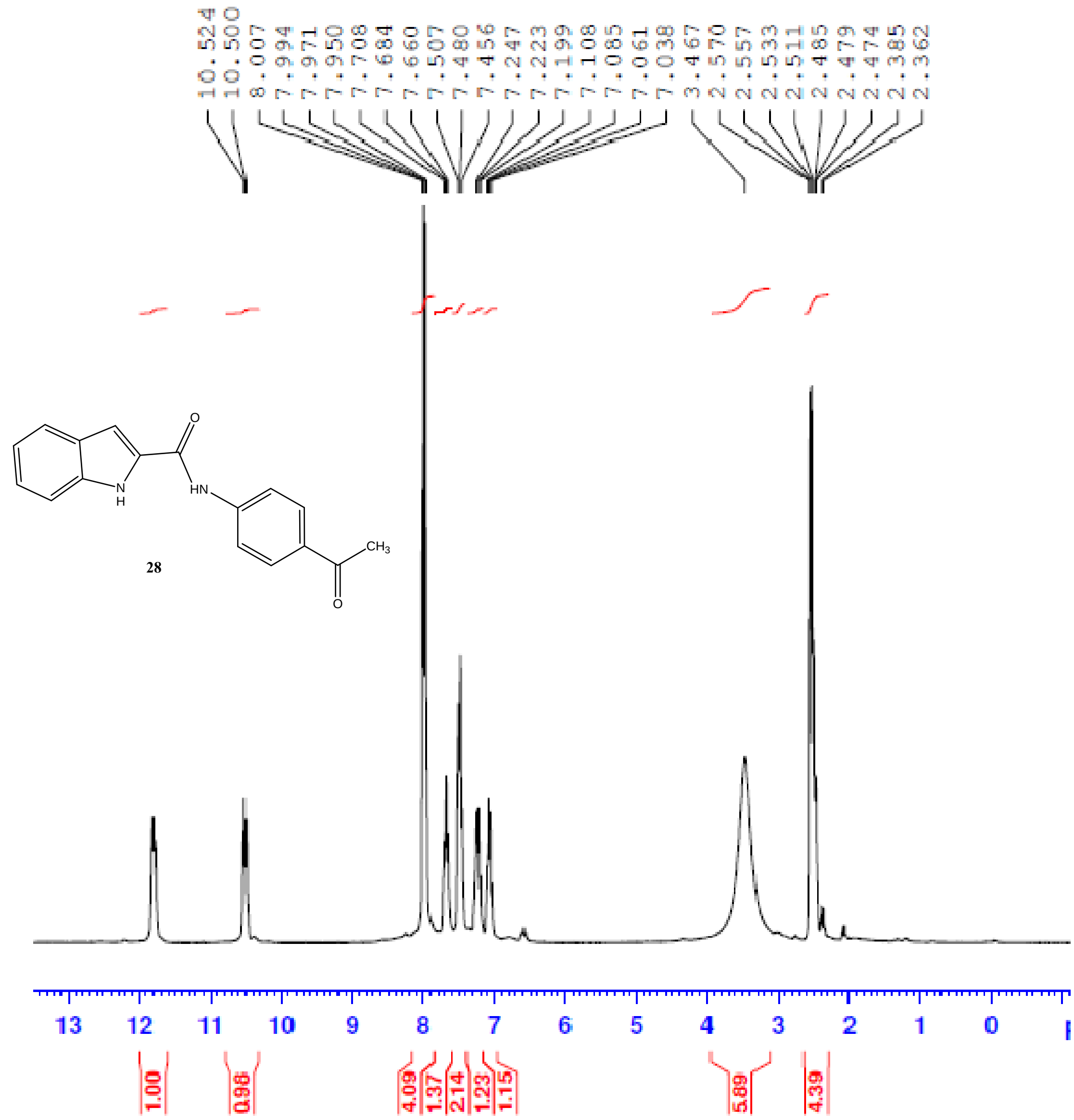
(¹H-NMR for C110)



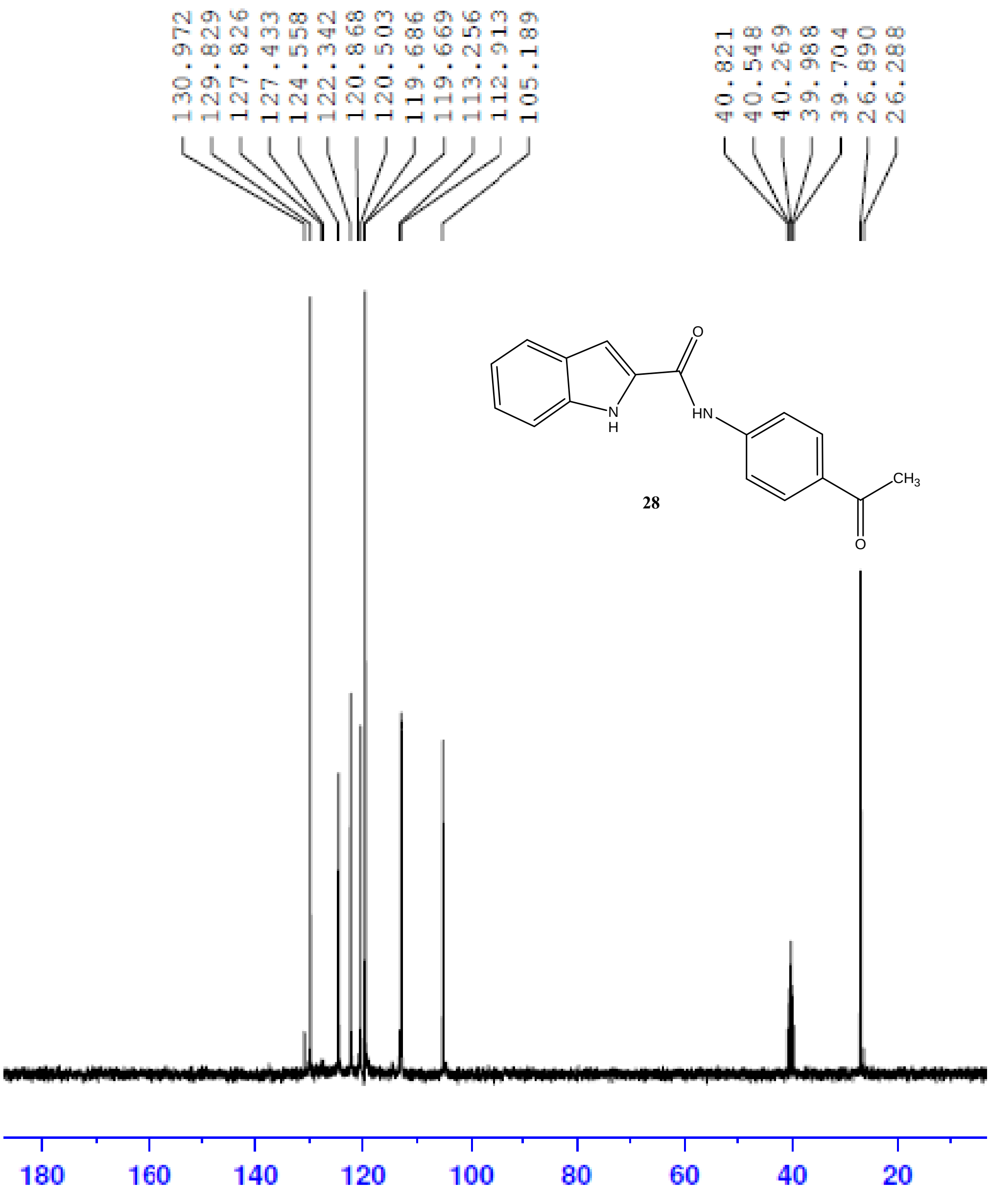
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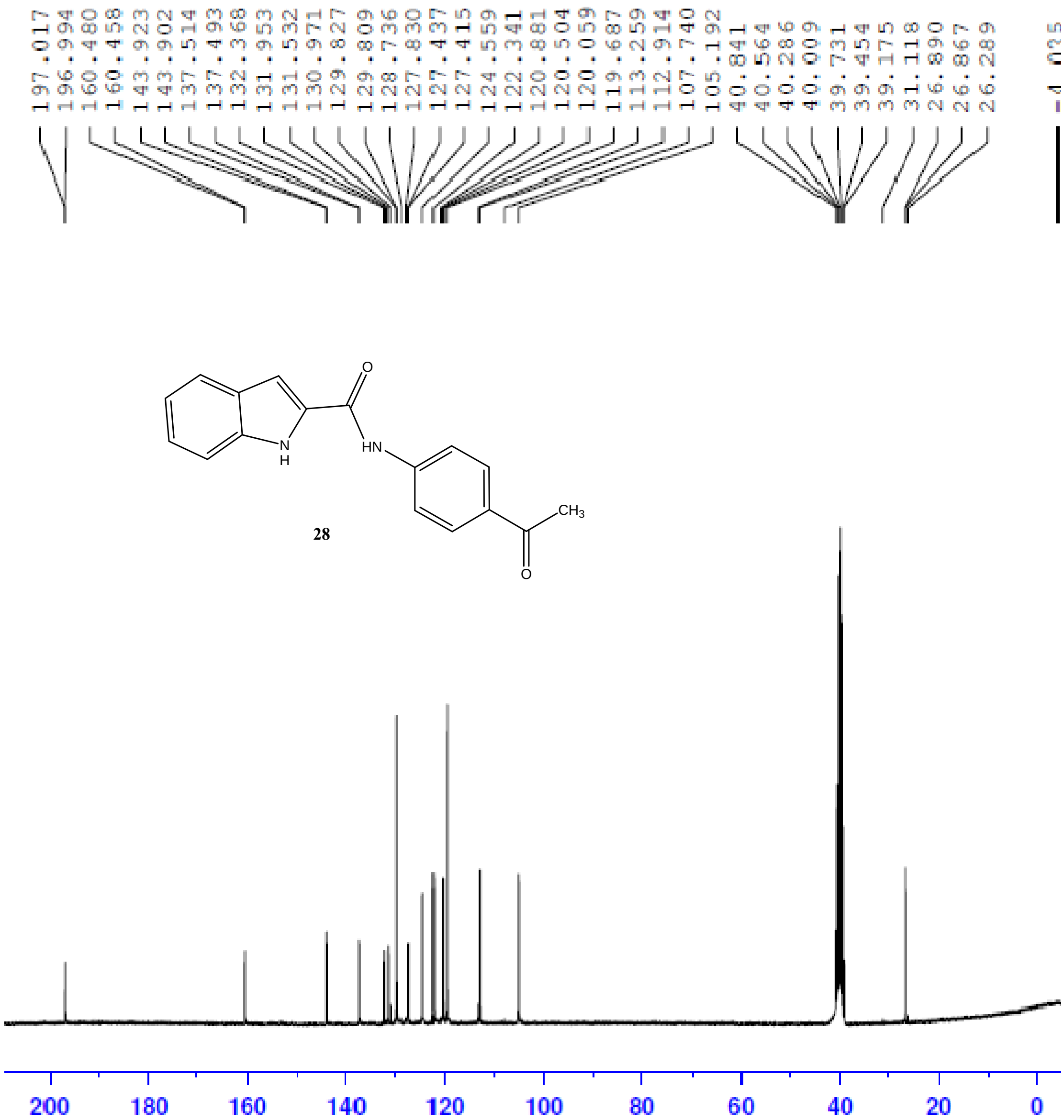
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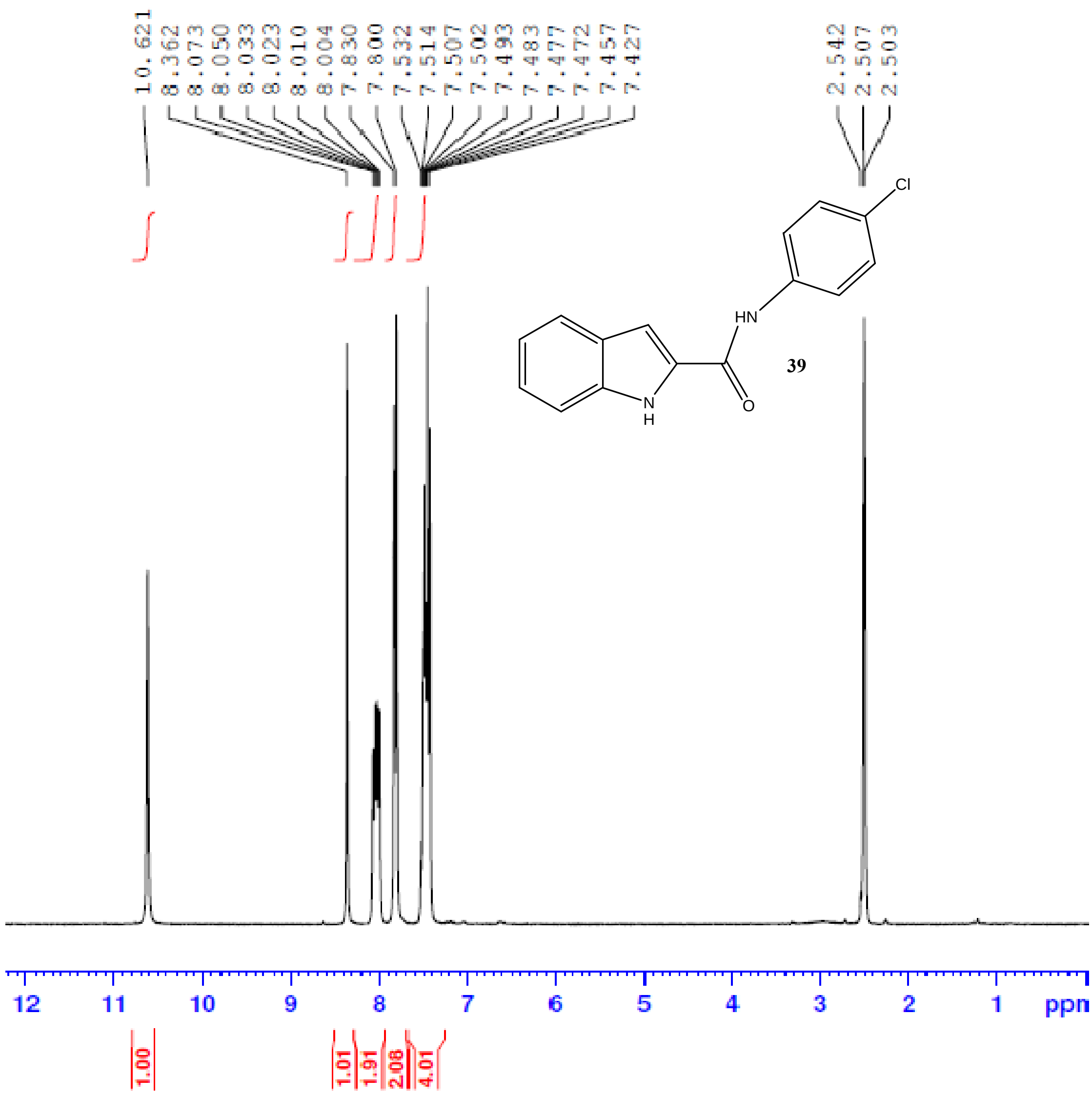
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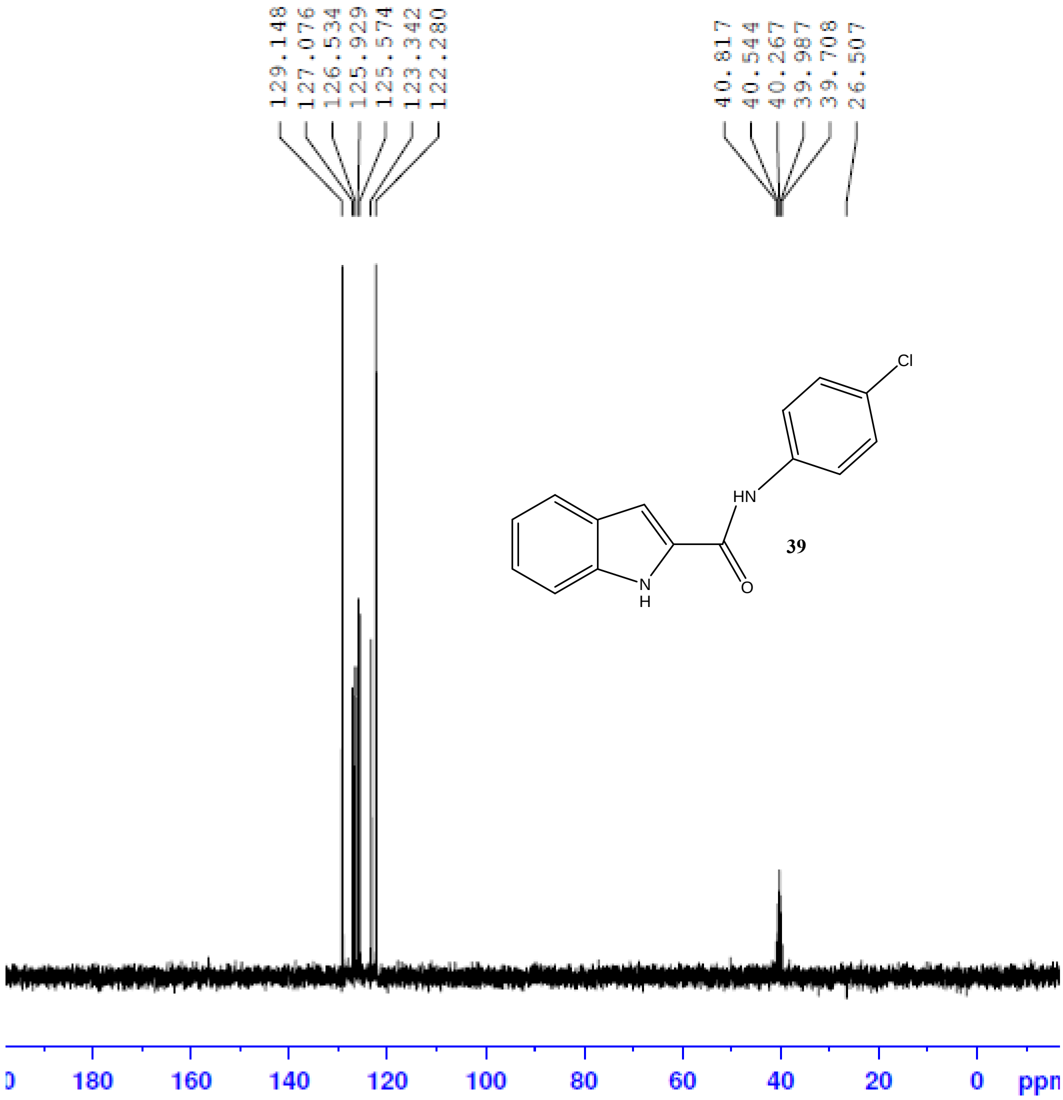
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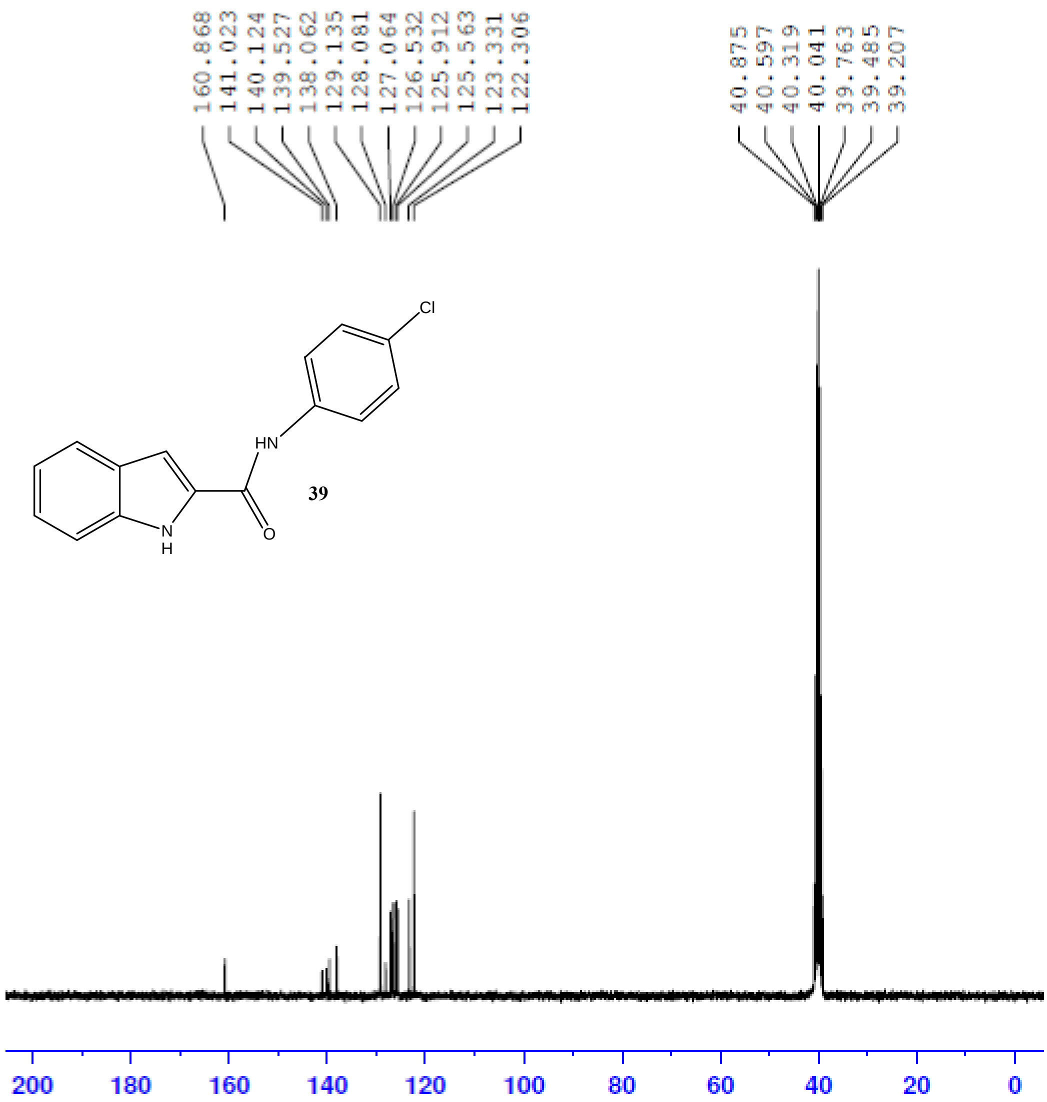
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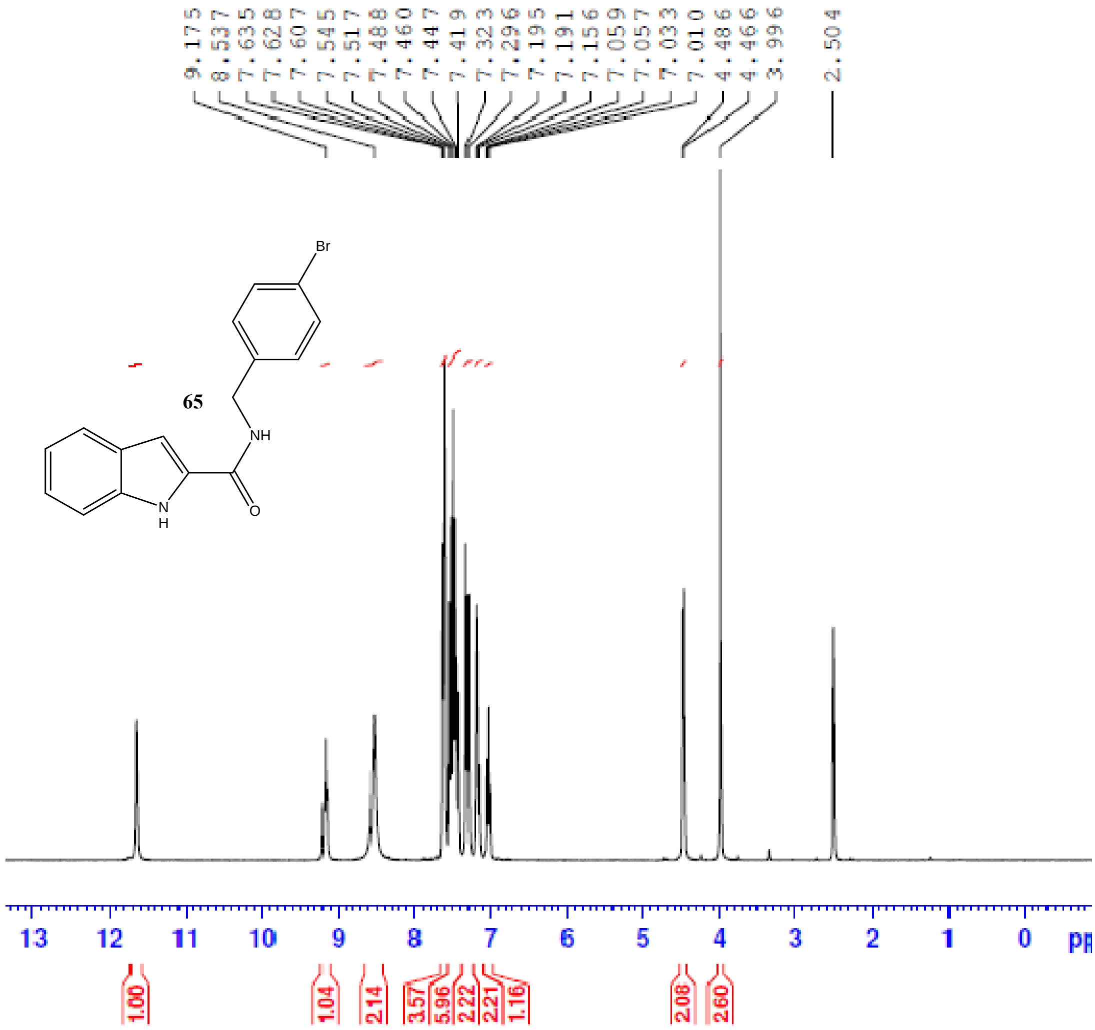
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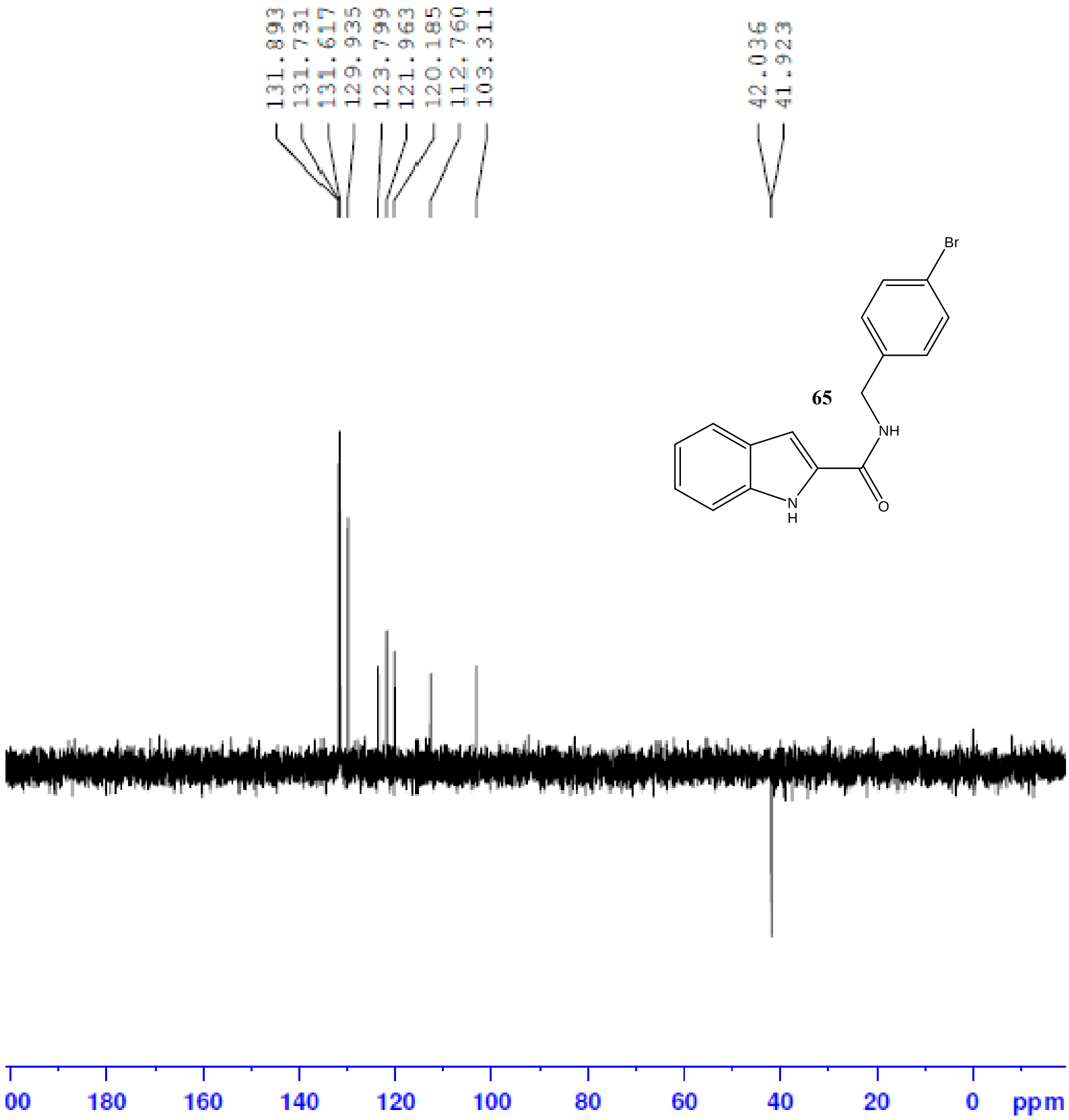
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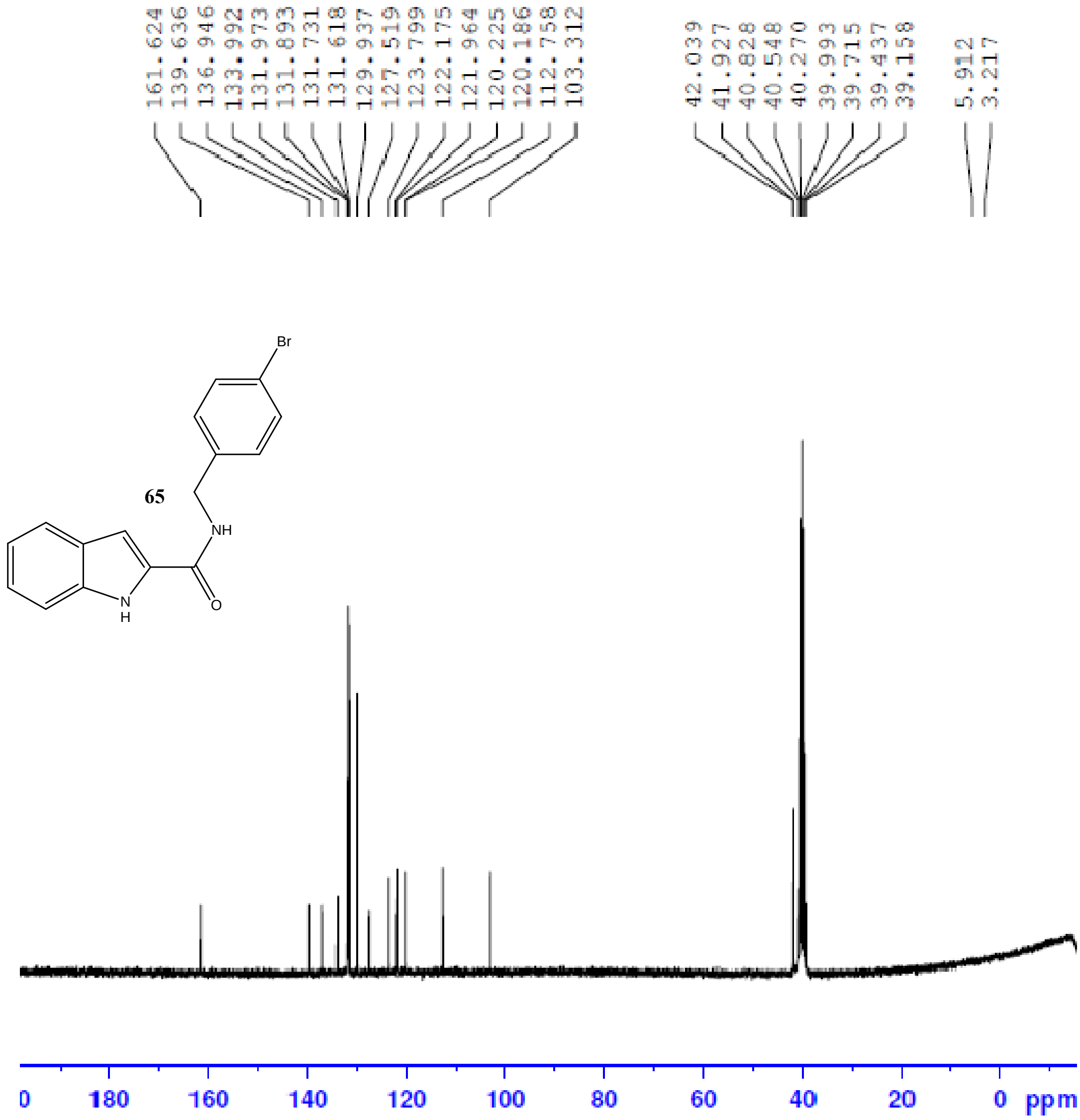
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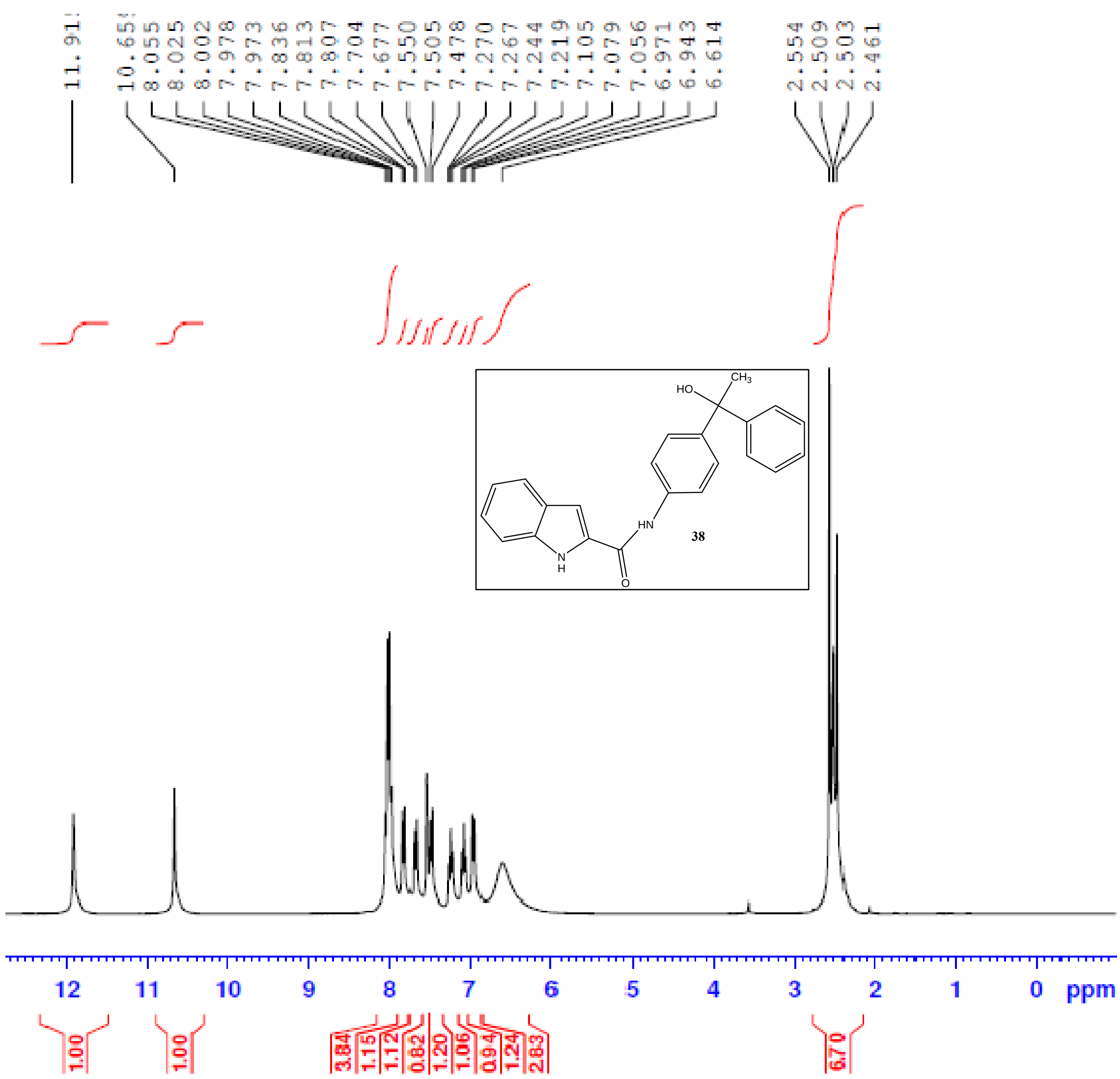
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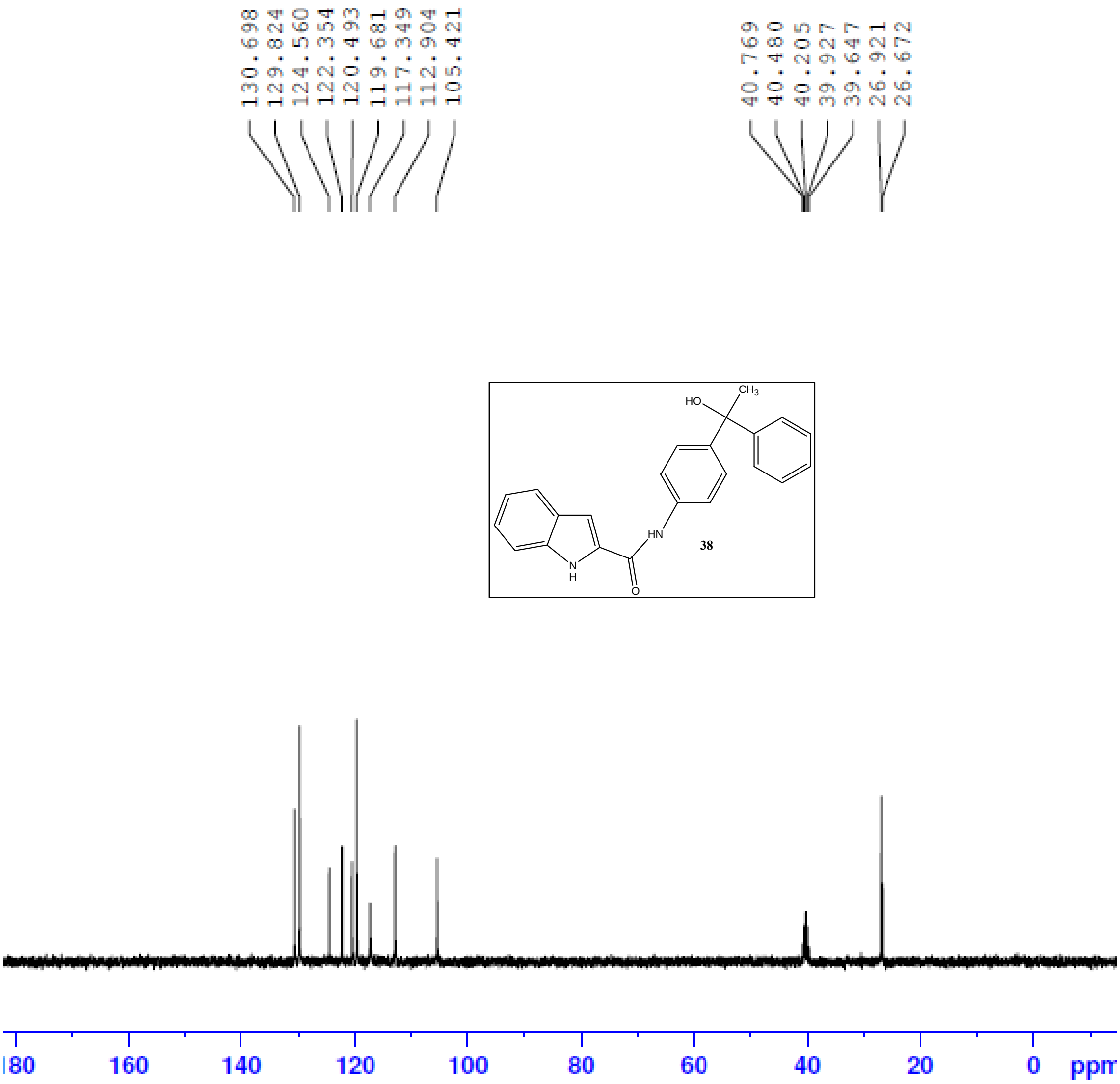
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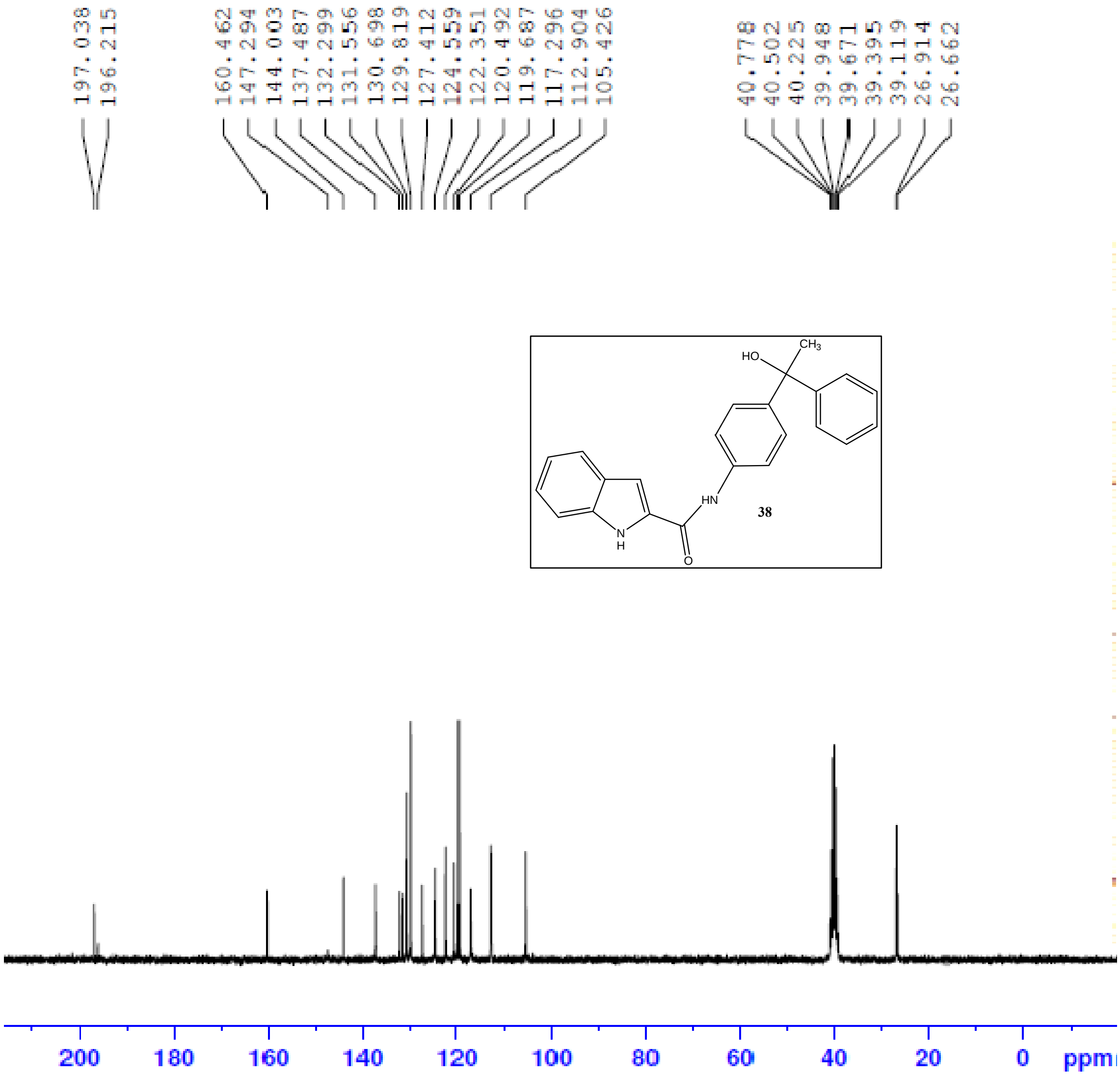
(^{13}C-NMR for C65)



(^{1}H-NMR for C36)



(Dept 135 for C36)



(^{13}C-NMR for C36)

Appendix II

A conversion table for the dose of the tested compounds in (mg) given for a rat weighing (250 g) which is equivalent to 57µM.

Compound number	Dose (mg/250g)	Compound number	Dose (mg/250g)
C6	19.40	C63	18.37
C8	21.36	C67	16.23
C10	19.40	C73	16.78
C14	20.20	C75	19.56
C16	21.91	C79	13.52
C18	20.88	C83	15.75
C20	20.88	C85	16.83
C22	21.80	C88	21.36
C24	22.85	C90	22.85
C26	26.18	C93	20.43
C28	15.86	C96	20.20
C30	15.86	C97	21.68
C32	15.86	C101	16.55
C36	20.32	C103	16.55
C38	22.11	C104	18.03
C47	18.60	C108	17.35
C51	16.03	C110	16.60
C53	15.12	C111	18.09
C57	17.23		

تصنيع مشتقات جديده من حلقيات غير متجانسه كاربوكساميد وتقيمها كمركبات خافضه للدهنيات ومضاده للتأكسد

اعداد
منال ممدوح النجداوي

المشرف
الدكتور يوسف محمد الحياري

المشرف المشارك
الدكتور طارق مصباح القرم

الملخص

يهدف هذا البحث إلى تحضير مشتقات جديدة من مجموعة الإندول - 2 – كاربوكساميد (المجموعة من G-A) وكذلك المجموعات المقابله بايرول وفيوران -2- كاربوكساميد (المجموعة H) لاختبار فعالياتها كمركبات مضادة للتأكسد وخافضة للدهنيات و فحصها على الجرذان باستعمال تريتون 1339– WR كنموذج على تركيز ثابت مقداره 57 ميكرومولار.
المركبات الجديده هي من مشتقات الإندول - 2 – كاربوكساميد: البنزوفينون (مجموعة A; 6, 8, 10, 12, 14, 16), الانثراكينون (مجموعة B; 18, 20, 22, 24, 26), اسيتوفينون (مجموعة C; 28, 30, 32, 34, 38,36), بنزين- انيلين (مجموعة D; 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63), بنزايل وفينيل ايثيل (مجموعة E; 65, 67, 69, 71, 73, 75), والهيتيروسايكل (مجموعة F; 77, 79, 81, 83), استبدالات الإندول (مجموعة G; 88, 89, 90, 93, 96, 97, 98), فيوران و البايرول -2- كاربوكساميد (مجموعة H; 101, 102, 103, 104, 108, 110, 111).
تم تحضير معظم هذه المركبات باعتماد الاقتران المباشر لاستر الإندول - 2 - كاوبوكسيلات أو الاسيل كلوريد مع الأمينات الأولية المناسبه في مذيب مثل: DMSO أو DMF $CHCl_3$ مع أو من دون القاعدة. بديلا عن ذلك ، تم استخدام الاندماج المباشر مع بعض المجموعات لإعطاء كميات بنسب اعلى. تم الحصول على معظم المركبات بنسب معقوله وتأكدت اشكالها بواسطة NMR, IR ، MS و EA.
بالنسبه للنشاط كخافض للدهنيات ، تم اختيار الجرعة على أساس دراسة السمية الحادة ممثلة بالمركب 6. تم تحضير الدهون بانواعها باستخدام جرعة واحدة من WR تريتون 1339 (250 ملغم / كغم ،) للجرذان. تم تحديد فعالية كل مركب من خلال قياس مستويات TG، TC، HDL و LDL باستخدام البنزافيبرات كمرجع بعد 8 ساعات.
وقد أظهرت المركبات 10 و 6 من مجموعة A ، انخفاضا كبيرا في مستويات الدهون الثلاثية (71 ٪) مقارنة مع الفئران المعالجه بالترايتون وحده. باستثناء مركب 20 ، أظهرت جميع مركبات 18, 20, 22, 24, 26 انخفاضا كبيرا في مستويات الدهون الثلاثية (65-71 ٪) مقارنة مع الفئران المعالجه بالترايتون وحده. وكانت المركبات من (مجموعة C، D، E و F) ، كلها غير قادرة على خفض مستويات الدهون الثلاثية على الفئران المعالجه بالترايتون وحده. وقد أظهرت مركبات

93, 90, 88 من مجموعة G ، انخفاضا كبيرا في مستويات الدهون الثلاثية (70-82 ٪) مقارنة مع الفئران المعالجه بالترايتون وحده. وقد أظهرت المركبات 110, 103, 104, 111 ، من المجموعة H ، انخفاضا كبيرا في مستويات الدهون الثلاثية (67-71 ٪) مقارنة مع الفئران المعالجه بالترايتون وحده.

وارتبط خفض الكولسترول الكلي مع انخفاض في نسبة الدهنيات القليلة الكثافة من المركبات 6 ، 18 ، 24 ، 26 ، 36 ، 88 ، 90 ، 103 ، 104 و 111 ، والذي هو الهدف من الأدوية التي تخفض الدهنيات في الدم. بالإضافة إلى ذلك ، المركبات 6 ، 10 ، 18 ، 22 ، 24 ، 26 ، 36 ، 38 ، 93 ، 88 ، 90 ، 110 ، 103 ، 104 و 111 ، زادت نسبة الدهنيات مرتفعة الكثافة (الكوليسترول الجيد) بشكل ملحوظ بعد 8 ساعات من اعطاء التريتون ، والذي يعرف لدوره الوقائي ضد تصلب الشرايينز

وخفض الدهون بواسطة المركبات 6 ، 10 ، 18 ، 22 ، 24 ، 26 ، 36 ، 38 ، 93 ، 88 ، 90 ، 110 ، 103 ، 104 و 111 ، في 57 ميكرومتر بعد الحقن تريتون 8 ساعات كان أكثر أهمية من الخفض الناجم عن بنزافيبرات.

الهيكل الشكلي للإندول و مستبدلاته له اهميه كبيره في قدرته على الارتباط مع المستقبلات باعطائه أو اخذه للرابطه الهيدروجينيه. مطلوب جزء محب للدهون في المركب الفعال ، والذي يتمثل من قبل اثنين من مجموعة فينيل ربما في الارتباط مع الأحماض الأمينية الموجوده في المستقبلات ، وبالاضافة لاعطاء المركب خاصية الانجذاب للدهون وليس الماء, كما ثبت من مركبات 36 و 38. نقطة جوهرية استنتجت من هذه الدراسة ، هو شكل المركبات المستهدفة أو التكوين المكاني ، الشكل الممتد اظهر فعاليه، في حين أن المركب على شكل V غير نشط او فعال.

وقد أظهرت المركبات 22 ، 57 ، 30 ، 55 و 59 في تثبيط نشاط DPPH بالنسب التاليه على التوالي (82%, 69.3%, 62.5%, 54.7%, 34.3%) ، وكانت الأكثر فعالية. وأظهر المركب 22 ، زيادة كبيرة في النشاط المتطرف الكسح بالإضافة إلى قدرته على خفض مستويات الدهون الذي قد يكون مرشحا واعدا للمركبات المحضره وقد يعتبر مركب مزدوج الفعاليه.